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(54) Title: MORPHOGEN-INDUCED MODULATION OF INFLAMMATORY RESPONSE

(57) Abstract

The present invention is directed to methods and compositions for alleviating tissue destructive effects associated with the inflammatory response to tissue injury in a mammal. The methods and compositions include administering a therapeutically effective concentration of a morphogen or morphogen-stimulating agent sufficient to alleviate immune cell-mediated tissue destruction.

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# MORPHOGEN-INDUCED MODULATION OF INFLAMMATORY RESPONSE

## Field of the Invention

The present invention relates generally to a method for modulating the inflammatory response induced in a mammal following tissue injury. More particularly, this invention relates to a method for alleviating immune-cell mediated tissue destruction associated with the inflammatory response.

## Background of the Invention

The body's inflammatory response to tissue injury can cause significant tissue destruction, leading to loss of tissue function. Damage to cells resulting from the effects of inflammatory response e.g., by immune-cell mediated tissue destruction, has been implicated as the cause of reduced tissue function or loss of tissue function in diseases of the joints (e.g., rheumatoid and osteo-arthritis) and of many organs, including the kidney, pancreas, skin, lung and heart. For example, glomular nephritis, diabetes, inflammatory bowel disease, vascular diseases such as atheroclerosis and vasculitis, and skin diseases such as psoriasis and dermatitis are believed to result in large part from unwanted acute inflammatory reaction and fibrosis. A number of these diseases, including arthritis, psoriasis and inflammatory bowel disease are considered to be chronic inflammatory diseases. The

damaged tissue also often is replaced by fibrotic tissue, e.g., scar tissue, which further reduces tissue function. Graft and transplanted organ rejection also is believed to be primarily due to the action of the body's immune/inflammatory response system.

The immune-cell mediated tissue destruction often follows an initial tissue injury or insult. The secondary damage, resulting from the inflammatory response, often is the source of significant tissue damage. Among the factors thought to mediate these damaging effects are those associated with modulating the body's inflammatory response following tissue injury, e.g., cytokines such as interleukin-I (IL-1) and tumor necrosis factor (TNF), and oxygen-derived free radicals such as superoxide anions. These humoral agents are produced by adhering neutrophilic leukocytes or by endothelial cells and have been identified at ischemic sites upon reperfusion. Moreover, TNF concentrations are increased in humans after myocardial infarction.

A variety of lung diseases are characterized by airway inflammation, including chronic bronchitis, emphysema, idiopathic pulmonary fibrosis and asthma. Another type of lung-related inflammation disorders are inflammatory diseases characterized by a generalized, wide-spread acute inflammatory response such as adult respiratory distress syndrome. Another dysfunction associated with the inflammatory response is that mounted in response to injury caused by hyperoxia, e.g., prolonged exposure to lethally high concentrations of $O_2$ (95-100% $O_2$). Similarly, reduced

blood flow to a tissue (and, therefore reduced or lack of oxygen to tissues), as described below, also can induce a primary tissue injury that stimulates the inflammatory response.

It is well known that damage occurs to cells in mammals which have been deprived of oxygen. In fact, the interruption of blood flow, whether partial (hypoxia) or complete (ischemia) and the ensuing inflammatory responses may be the most important cause of coagulative necrosis or cell death in human disease. The complications of atherosclerosis, for example, are generally the result of ischemic cell injury in the brain, heart, small intestines, kidneys, and lower extremities. Highly differentiated cells, such as the proximal tubular cells of the kidney, cardiac myocytes, and the neurons of the central nervous system, all depend on aerobic respiration to produce ATP, the energy necessary to carry out their specialized functions. When ischemia limits the oxygen supply and ATP is depleted, the affected cells may become irreversibly injured. The ensuing inflammatory responses to this initial injury provide additional insult to the affected tissue. Examples of such hypoxia or ischemia are the partial or total loss of blood supply to the body as a whole, an organ within the body, or a region within an organ, such as occurs in cardiac arrest, pulmonary embolus, renal artery occlusion, coronary occlusion or occlusive stroke.

The tissue damage associated with ischemia-reperfusion injury is believed to comprise both the initial cell damage induced by the deprivation of oxygen to the cell and its subsequent recirculation, as well as the damage caused by the body's response to

this initial damage. It is thought that reperfusion injury may result in dysfunction to the endothelium of the vasculature as well as injury to the surrounding tissue. In idiopathic pulmonary fibrosis, for example, scar tissue accumulates on the lung tissue lining, inhibiting the tissue's elasticity. The tissue damage associated with hyperoxia injury is believed to follow a similar mechanism, where the initial damage is mediated primarily through the presence of toxic oxygen metabolites followed by an inflammatory response to this initial injury.

Similarly, tissues and organs for transplantation also are subject to the tissue destructive effects associated with the recipient host body's inflammatory response following transplantation. It is currently believed that the initial destructive response is due in large part to reperfusion injury to the transplanted organ after it has been transplanted to the organ recipient.

Accordingly, the success of organ or tissue transplantation depends greatly on the preservation of the tissue activity (e.g., tissue or organ viability) at the harvest of the organ, during storage of the harvested organ, and at transplantation. To date, preservation of organs such as lungs, pancreas, heart and liver remains a significant stumbling block to the successful transplantation of these organs. U.S. Patent No. 4,952,409 describes a superoxide dismutase-containing liposome to inhibit reperfusion injury. U.S. Patent No. 5,002,965 describes the use of ginkolides, known platelet activating factor antagonists, to inhibit reperfusion injury. Both of these factors are described working primarily by

inhibiting the release of and/or inhibiting the damaging effects of free oxygen radicals. A number of patents also have issued on the use of immunosuppressants for inhibiting graft rejection. A representative listing includes U.S. Patent Nos. 5,104,858, 5,008,246 and 5,068,323. A significant problem with many immunosuppressants is their low therapeutic index, requiring the administration of high doses that can have significant toxic side effects.

Rheumatoid and osteoarthritis are prevalent diseases characterized by chronic inflammation of the synovial membrane lining the afflicted joint. A major consequence of chronic inflammatory joint disease (e.g., rheumatoid arthritis) and degenerative arthritis (e.g., osteoarthritis) is loss of function of those affected joints. This loss of function is due primarily to destruction of the major structural components of the joint, cartilage and bone, and subsequent loss of the proper joint anatomy. As a consequence of chronic disease, joint destruction ensues and can lead to irreversible and permanent damage to the joint and loss of function. Current treatment methods for severe cases of rheumatoid arthritis typically include the removal of the synovial membrane, e.g., synovectomy. Surgical synovectomy has many limitations, including the risk of the surgical procedure itself, and the fact that a surgeon often cannot remove all of the diseased membrane. The diseased tissue remaining typically regenerates, causing the same symptoms which the surgery was meant to alleviate.

Psoriasis is a chronic, recurrent, scaling skin disease of unknown etiology characterized by chronic inflammation of the skin. Erythematous eruptions, often in papules or plaques, and usually having a white silvery scale, can affect any part of the skin, but most commonly affect the scalp, elbows, knees and lower back. The disease usually occurs in adults, but children may also be affected. Patients with psoriasis have a much greater incidence of arthritis (psoraitic arthritis), and generalized exfoliation and even death can threaten afflicted individuals.

Current therapeutic regimens include topical or intralesional application of corticosteroids, topical administration of keratolytics, and use of tar and UV light on affected areas. No single therapy is ideal, and it is rare for a patient not to be treated with several alternatives during the relapsing and remitting course of the disease. Whereas systematic treatment can induce prompt resolution of psoriatic lesions, suppression often requires ever-increasing doses, sometimes with toxic side effect, and tapering of therapy may result in rebound phenomena with extensions of lesions, possibly to exfoliation.

Inflammatory bowel disease (IBD) describes a class of clinical disorders of the gastrointestinal mucosa characterized by chronic inflammation and severe ulceration of the mucosa. The two major diseases in this classification are ulcerative colitis and regional enteritis (Crohn's Disease). Like oral mucositis, the diseases classified as IBD are associated with severe mucosal ulceration (frequently penetrating the wall of the bowel and forming strictures and fistulas), severe mucosal and submucosal inflammation and edema, and

fibrosis (e.g., scar tissue formation which interferes with the acid protective function of the gastrointestinal lining.) Other forms of IBD include regional ileitis and proctitis. Clinically, patients with fulminant IBD can be severely ill with massive diarrhea, blood loss, dehydration, weight loss and fever. The prognosis of the disease is not good and frequently requires resection of the diseased tissue.

Therefore, an object of the present invention is to provide a method for protecting mammalian tissue, particularly human tissue, from the damage associated with the inflammatory response following a tissue injury. The inflammatory reaction may be in response to an initial tissue injury or insult. The original injury may be chemically, mechanically, biologically or immunologically related. Another object is to provide methods and compositions for protecting tissue from the tissue destructive effects associated with chronic inflammatory diseases, including arthritis (e.g., reheumatoid or osteoarthritis), psoriatic arthritis, psoriasis and dermatitis, inflammatory bowel disease and other autoimmune diseases. Yet another object is to provide methods and compositions for enhancing the viability of mammalian tissues and organs to be transplanted, including protecting the transplanted organs from immune cell-mediated tissue destruction, such as the tissue damage associated with ischemia-reperfusion injury. This tissue damage may occur during donor tissue or organ harvesting and transport, as well as following initiation of blood flow after transplantation of the organ or tissue in the recipient host.

Another object of the invention is to provide a method for alleviating tissue damage associated with ischemic-reperfusion injury in a mammal following a deprivation of oxygen to a tissue in the mammal. Other objects of the present invention include providing a method for alleviating tissue damage associated with ischemic-reperfusion injury in a human which has suffered from hypoxia or ischemia following cardiac arrest, pulmonary embolus, renal artery occlusion, coronary occlusion or occlusive stroke. A further object is to provide a method for alleviating tissue damage associated with hyperoxia-induced tissue injury, e.g., lethally high oxygen concentrations.

Still another object of the invention is to provide a method for modulating inflammatory responses in general, particularly those induced in a human following tissue injury.

These and other objects and features of the invention will be apparent from the description, drawings and claims which follow.

## Summary of the Invention

The present invention provides a method for alleviating the tissue destructive effects associated with activation of the inflammatory response following tissue injury. The method comprises the step of providing to the affected tissue a therapeutically effective concentration of a morphogenic protein ("morphogen", as defined herein) upon tissue injury or in anticipation of tissue injury, sufficient to substantially inhibit or reduce the tissue destructive effects of the inflammatory response.

In one aspect, the invention features compositions and therapeutic treatment methods that comprise the step of administering to a mammal a therapeutically effective amount of a morphogenic protein ("morphogen"), as defined herein, upon injury to a tissue, or in anticipation of such injury, for a time and at a concentration sufficient to inhibit the tissue destructive effects associated with the body's inflammatory response, including repairing damaged tissue, and/or inhibiting additional damage thereto.

In another aspect, the invention features compositions and therapeutic treatment methods for protecting tissues and organs from the tissue destructive effects of the inflammatory response which include administering to the mammal, upon injury to a tissue or in anticipation of such injury, a compound that stimulates <u>in vivo</u> a therapeutically effective concentration of an endogenous morphogen within the body of the mammal sufficient to protect the tissue from the tissue destructive effects associated with the inflammatory response, including repairing damaged

tissue and/or inhibiting additional damage thereto. These compounds are referred to herein as morphogen-stimulating agents, and are understood to include substances which, when administered to a mammal, act on cells of tissue(s) or organ(s) that normally are responsible for, or capable of, producing a morphogen and/or secreting a morphogen, and which cause the endogenous level of the morphogen to be altered. The agent may act, for example, by stimulating expression and/or secretion of an endogenous morphogen.

As embodied herein, the term "ischemic-reperfusion injury" refers to the initial damage associated with oxygen deprivation of a cell and the subsequent damage associated with the inflammatory response when the cell is resupplied with oxygen. As embodied herein, the term "hyperoxia-induced injury" refers to the tissue damage associated with prolonged exposure to lethally high doses of oxygen, e.g., greater than 95% $O_2$, including the tissue damage associated with the inflammatory response to the toxically high oxygen dose. Accordingly, as used herein, "toxic oxygen concentrations" refers to the tissue damage associated withthe injury induced by both lethally low oxygen concentrations of oxygen (including a complete lack of oxygen), and by lethally high oxygen concentrations. The expression "alleviating" means the protection from, reduction of and/or elimination of undesired tissue destruction, particularly immune cell-mediated tissue destruction. The tissue destruction may be in response to an initial tissue injury, which may be mechanical, chemical or immunological in origin. The expression "enhance the viability of" living tissues or organs, as used herein, means protection from, reduction of and/or elimination of reduced or lost tissue or organ function

as a result of tissue death, particularly immune cell-mediated tissue death. "Transplanted" living tissue encompasses both tissue transplants (e.g., as in the case of bone marrow transplants) and tissue grafts. Finally, a "free oxygen radical inhibiting agent" means a molecule capable of inhibiting the release of and/or inhibiting tissue damaging effects of free oxygen radicals.

In one embodiment of the invention, the invention provides methods and compositions for alleviating the ischemic-reperfusion injury in mammalian tissue resulting from a deprivation of, and subsequent reperfusion of, oxygen to the tissue. In another embodiment, the invention provides a method for alleviating the tissue-destructive effects associated with hyperoxia. In still another embodiment of the invention, the invention provides methods and compositions for maintaining the viability of tissues and organs, particularly living tissues and organs to be transplanted, including protecting them from ischemia-reperfusion injury. In still another embodiment, the invention provides methods for protecting tissues and organs from the tissue destructive effects of chronic inflammatory diseases, such as arthritis, psoriasis, dermatitis, including contact dermatitis, IBD and other chronic inflammatory diseases of the gastrointestinal tract, as well as the tissue destructive effects associated with other, known autoimmune diseases, such as diabetes, multiple sclerosis, amyotrophic lateral sclerosis (ALS), and other autoimmune neurodegenerative diseases.

In one aspect of the invention, the morphogen is provided to the damaged tissue following an initial injury to the tissue. The morphogen may be provided directly to the tissue, as by injection to the damaged tissue site or by topical administration, or may be provided indirectly, e.g., systemically by oral or parenteral means. Alternatively, as described above, an agent capable of stimulating endogenous morphogen expression and/or secretion may be administered to the mammal. Preferably, the agent can stimulate an endogenous morphogen in cells associated with the damaged tissue. Alternatively, morphogen expression and/or secretion may be stimulated in a distant tissue and the morphogen transported to the damaged tissue by the circulatory system.

In another aspect of the invention, the morphogen is provided to tissue at risk of damage due to immune cell-mediated tissue destruction. Examples of such tissues include tissue grafts and tissue or organ transplants, as well as any tissue or organ about to undergo a surgical procedure or other clinical procedure likely to either inhibit blood flow to the tissue or otherwise induce an inflammatory response. Here the morphogen or morphogen-stimulating agent preferably is provided to the patient prior to induction of the injury, e.g., as a prophylactic, to provide a cyto-protective effect to the tissue at risk.

Where the tissue at risk comprises a tissue or organ to be transplanted, the tissue or organ to be transplanted preferably is exposed to a morphogen prior to transplantation. Most preferably, the tissue or organ is exposed to the morphogen prior to its removal from the donor, by providing the donor with a

composition comprising a morphogen or morphogen-stimulating agent. Alternatively or, in addition, once removed from the donor, the organ or tissue is placed in a preservation solution containing a morphogen or morphogen-stimulating agent. In addition, the recipient also preferably is provided with a morphogen or morphogen-stimulating agent just prior to, or concommitant with, transplantation. In all cases, the morphogen or morphogen-stimulating agent may be administered directly to the tissue at risk, as by injection or topical administration to the tissue, or it may be provided systemically, either by oral or parenteral administration.

The morphogens described herein are envisioned to be useful in enhancing viability of any organ or living tissue to be transplanted. The morphogens may be used to particular advantage in lung, heart, liver, kidney or pancreas transplants, as well as in transplantation and/or grafting of bone marrow, skin, gastrointestinal mucosa, and other living tissues.

Where the patient suffers from a chronic inflammatory disease, such as diabetes, arthritis, psoriasis, IBD, and the like, the morphogen or morphogen-stimulating agent preferably is administered at regular intervals as a prophylactic, to prevent and/or inhibit the tissue damage normally associated with the disease during flare periods. As above, the morphogen or morphogen-stimulating agent may be provided directly to the tissue at risk, for example by injection or by topical administration, or indirectly, as by systemic e.g., oral or parenteral administration.

Among the morphogens useful in this invention are proteins originally identified as osteogenic proteins, such as the OP-1, OP-2 and CBMP2 proteins, as well as amino acid sequence-related proteins such as DPP (from Drosophila), Vgl (from Xenopus), Vgr-1 (from mouse, see U.S. 5,011,691 to Oppermann et al.), GDF-1 (from mouse, see Lee (1991) PNAS 88:4250-4254), all of which are presented in Table II and Seq. ID Nos.5-14), and the recently identified 60A protein (from Drosophila, Seq. ID No. 24, see Wharton et al. (1991) PNAS 88:9214-9218.) The members of this family, which include members of the TGF-β super-family of proteins, share substantial amino acid sequence homology in their C-terminal regions. The proteins are translated as a precursor, having an N-terminal signal peptide sequence, typically less than about 30 residues, followed by a "pro" domain that is cleaved to yield the mature sequence. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be predicted in a given sequence using the method of Von Heijne ((1986) Nucleic Acids Research 14:4683-4691.) Table I, below, describes the various morphogens identified to date, including their nomenclature as used herein, their Seq. ID references, and publication sources for the amino acid sequences for the full length proteins not included in the Seq. Listing. The disclosure of these publications is incorporated herein by reference.

TABLE I

"OP-1" Refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human

OP-1 ("hOP-1", Seq. ID No. 5, mature protein amino acid sequence), or mouse OP-1 ("mOP-1", Seq. ID No. 6, mature protein amino acid sequence.) The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. Id Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.) The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).

"OP-2" refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-2 protein, including allelic and species variants thereof, e.g., human OP-2 ("hOP-2", Seq. ID No. 7, mature protein amino acid sequence) or mouse OP-2 ("mOP-2", Seq. ID No. 8, mature protein amino acid sequence). The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 7 and 8. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 20 and 21 (hOP2) and Seq. ID Nos. 22 and 23 (mOP2.) The mature proteins are defined essentially by residues 264-402 (hOP2) and 261-399 (mOP2). The "pro"

regions of the proteins, cleaved to yield the mature, morphogenically active proteins likely are defined essentially by residues 18-263 (hOP2) and residues 18-260 (mOP2). (Another cleavage site also occurs 21 residues upstream for both OP-2 proteins.)

"CBMP2" refers generically to the morphogenically active proteins expressed from a DNA sequence encoding the CBMP2 proteins, including allelic and species variants thereof, e.g., human CBMP2A ("CBMP2A(fx)", Seq ID No. 9) or human CBMP2B DNA ("CBMP2B(fx)", Seq. ID No. 10). The amino acid sequence for the full length proteins, referred to in the literature as BMP2A and BMP2B, or BMP2 and BMP4, appear in Wozney, et al. (1988) Science 242:1528-1534. The pro domain for BMP2 (BMP2A) likely includes residues 25-248 or 25-282; the mature protein, residues 249-396 or 283-396. The pro domain for BMP4 (BMP2B) likely includes residues 25-256 or 25-292; the mature protein, residues 257-408 or 293-408.

"DPP(fx)" refers to protein sequences encoded by the Drosophila DPP gene and defining the conserved seven cysteine skeleton (Seq. ID No. 11). The amino acid sequence for the full length protein appears in Padgett, et

al (1987) <u>Nature</u> <u>325</u>: 81-84. The pro domain likely extends from the signal peptide cleavage site to residue 456; the mature protein likely is defined by residues 457-588.

"Vgl(fx)" refers to protein sequences encoded by the Xenopus Vgl gene and defining the conserved seven cysteine skeleton (Seq. ID No. 12). The amino acid sequence for the full length protein appears in Weeks (1987) <u>Cell</u> <u>51</u>: 861-867. The prodomain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360.

"Vgr-1(fx)" refers to protein sequences encoded by the murine Vgr-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 13). The amino acid sequence for the full length protein appears in Lyons, et al, (1989) <u>PNAS</u> <u>86</u>: 4554-4558. The prodomain likely extends from the signal peptide cleavage site to residue 299; the mature protein likely is defined by residues 300-438.

"GDF-1(fx)" refers to protein sequences encoded by the human GDF-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 14). The cDNA and encoded amino sequence for the full length protein is

provided in Seq. ID. No. 32. The prodomain likely extends from the signal peptide clavage site to residue 214; the mature protein likely is defined by residues 215-372.

"60A" refers generically to the morphogenically active proteins expressed from part or all of a DNA sequence (from the Drosophila 60A gene) encoding the 60A proteins (see Seq. ID No. 24 wherein the cDNA and encoded amino acid sequence for the full length protein is provided). "60A(fx)" refers to the protein sequences defining the conserved seven cysteine skeleton (residues 354 to 455 of Seq. ID No. 24.) The prodomain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455.

"BMP3(fx)" refers to protein sequences encoded by the human BMP3 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 26). The amino acid sequence for the full length protein appears in Wozney et al. (1988) Science 242: 1528-1534. The pro domain likely extends from the signal peptide cleavage site to residue 290; the mature protein likely is defined by residues 291-472.

"BMP5(fx)" refers to protein sequences encoded by the human BMP5 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 27). The amino acid sequence for the full length protein appears in Celeste, et al. (1991) PNAS 87: 9843-9847. The pro domain likely extends from the signal peptide cleavage site to residue 316; the mature protein likely is defined by residues 317-454.

"BMP6(fx)" refers to protein sequences encoded by the human BMP6 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 28). The amino acid sequence for the full length protein appears in Celeste, et al. (1990) PNAS 87: 9843-5847. The pro domain likely includes extends from the signal peptide cleavage site to residue 374; the mature sequence likely includes residues 375-513.

The OP-2 proteins have an additional cysteine residue in this region (e.g., see residue 41 of Seq. ID Nos. 7 and 8), in addition to the conserved cysteine skeleton in common with the other proteins in this family. The GDF-1 protein has a four amino acid insert within the conserved skeleton (residues 44-47 of Seq. ID No. 14) but this insert likely does not interfere with the relationship of the cysteines in the folded structure. In addition, the CBMP2 proteins are missing one amino acid residue within the cysteine skeleton.

The morphogens are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this invention (e.g., as heterodimers). Thus, as defined herein, a morphogen is a dimeric protein comprising a pair of polypeptide chains, wherein each polypeptide chain comprises at least the C-terminal six cysteine skeleton defined by residues 43-139 of Seq. ID No. 5, including functionally equivalent arrangements of these cysteines (e.g., amino acid insertions or deletions which alter the linear arrangement of the cysteines in the sequence but not their relationship in the folded structure), such that, when the polypeptide chains are folded, the dimeric protein species comprising the pair of polypeptide chains has the appropriate three-dimensional structure, including the appropriate intra- or inter-chain disulfide bonds such that the protein is capable of acting as a morphogen as defined herein. Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells, including the "redifferentiation" of transformed cells. In addition, it is also anticipated that these morphogens are capable of inducing redifferentiation of committed cells under appropriate environmental conditions.

In one preferred aspect, the morphogens of this invention comprise one of two species of generic amino acid sequences: Generic Sequence 1 (Seq. ID No. 1) or Generic Sequence 2 (Seq. ID No. 2); where each Xaa

indicates one of the 20 naturally-occurring L-isomer, α-amino acids or a derivative thereof. Generic Sequence 1 comprises the conserved six cysteine skeleton and Generic Sequence 2 comprises the conserved six cysteine skeleton plus the additional cysteine identified in OP-2 (see residue 36, Seq. ID No. 2). In another preferred aspect, these sequences further comprise the following additional sequence at their N-terminus:

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)
1 5

Preferred amino acid sequences within the foregoing generic sequences include: Generic Sequence 3 (Seq. ID No. 3), Generic Sequence 4 (Seq. ID No. 4), Generic Sequence 5 (Seq. ID No. 30) and Generic Sequence 6 (Seq. ID No. 31), listed below. These Generic Sequences accommodate the homologies shared among the various preferred members of this morphogen family identified in Table II, as well as the amino acid sequence variation among them. Specifically, Generic Sequences 3 and 4 are composite amino acid sequences of the following proteins presented in Table II and identified in Seq. ID Nos. 5-14: human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (from mouse, Seq. ID No. 14.) The generic sequences include both the amino acid identity shared by the sequences in Table II, as well as alternative residues for the variable positions within the sequence. Note that

these generic sequences allow for an additional cysteine at position 41 or 46 in Generic Sequences 3 or 4, respectively, providing an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and contain certain critical amino acids which influence the tertiary structure of the proteins.

<u>Generic Sequence 3</u>

Leu Tyr Val Xaa Phe
1 5

Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
10

Xaa Ala Pro Xaa Gly Xaa Xaa Ala
15 20

Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
25 30

Xaa Pro Xaa Xaa Xaa Xaa Xaa
35

Xaa Xaa Xaa Asn His Ala Xaa Xaa
40 45

Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
50

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
55 60

Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
65

Xaa Xaa Xaa Leu Xaa Xaa Xaa

70 75

Xaa Xaa Xaa Xaa Val Xaa Leu Xaa

80

Xaa Xaa Xaa Xaa Met Xaa Val Xaa

85 90

Xaa Cys Gly Cys Xaa

95

wherein each Xaa is independently selected from a group of one or more specified amino acids defined as follows: "Res." means "residue" and Xaa at res.4 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser or Lys); Xaa at res.7 = (Asp or Glu); Xaa at res.8 = (Leu or Val); Xaa at res.11 = (Gln, Leu, Asp, His or Asn); Xaa at res.12 = (Asp, Arg or Asn); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.18 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Leu or Gln); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, Tyr, Asp or Gln); Xaa at res.28 = (Glu, Lys, Asp or Gln); Xaa at res.30 = (Ala, Ser, Pro or Gln); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu or Val); Xaa at res.34 = (Asn, Asp, Ala or Thr); Xaa at res.35 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn or Ser); Xaa at res.39 = (Ala, Ser or Gly); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile or Val); Xaa at res.45 = (Val or Leu); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His or Asn); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa

at res.52 = (Ile, Met, Asn, Ala or Val); Xaa at res.53 = (Asn, Lys, Ala or Glu); Xaa at res.54 = (Pro or Ser); Xaa at res.55 = (Glu, Asp, Asn, or Gly); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys or Leu); Xaa at res.60 = (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr or Ala); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser or Asp); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr or Val); Xaa at res.71 = (Ser or Ala); Xaa at res.72 = (Val or Met); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr or Leu); Xaa at res.76 = (Asp or Asn); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn or Tyr); Xaa at res.79 = (Ser, Asn, Asp or Glu); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile or Val); Xaa at res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln or His); Xaa at res.86 = (Tyr or His); Xaa at res.87 = (Arg, Gln or Glu); Xaa at res.88 = (Asn, Glu or Asp); Xaa at res.90 = (Val, Thr or Ala); Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at res.93 = (Ala, Gly or Glu); and Xaa at res.97 = (His or Arg);

Generic Sequence 4

```
Cys Xaa Xaa Xaa Xaa Leu Tyr Val Xaa Phe
 1               5                  10
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                 15
Xaa Ala Pro Xaa Gly Xaa Xaa Ala
 20                  25
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
        30                  35
```

Xaa Pro Xaa Xaa Xaa Xaa Xaa
40
Xaa Xaa Xaa Asn His Ala Xaa Xaa
45 50
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
55
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
60 65
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
70
Xaa Xaa Xaa Leu Xaa Xaa Xaa
75 80
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
85
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
90 95
Xaa Cys Gly Cys Xaa
100

wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the following: "Res." means "residue" and Xaa at res.2 = (Lys or Arg); Xaa at res.3 = (Lys or Arg); Xaa at res.4 = (His or Arg); Xaa at res.5 = (Glu, Ser, His, Gly, Arg or Pro); Xaa at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg, Gln, Ser or Lys); Xaa at res.12 = (Asp or Glu); Xaa at res.13 = (Leu or Val); Xaa at res.16 = (Gln, Leu, Asp, His or Asn); Xaa at res.17 = (Asp, Arg, or Asn); Xaa at res.19 = (Ile or Val); Xaa at res.20 = (Ile or Val); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Leu, or Gln); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp or Gln); Xaa at res.33 = Glu, Lys, Asp or Gln); Xaa at res.35 = (Ala, Ser or Pro); Xaa at res.36 = (Phe, Leu or Tyr); Xaa at res.38 = (Leu or Val); Xaa at res.39 =

(Asn, Asp, Ala or Thr); Xaa at res.40 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.41 = (Tyr, Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at res.44 = (Ala, Ser or Gly); Xaa at res.45 = (Thr, Leu or Ser); Xaa at res.49 = (Ile or Val); Xaa at res.50 = (Val or Leu); Xaa at res.51 = (Gln or Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.54 = (Val or Met); Xaa at res.55 = (His or Asn); Xaa at res.56 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn, Ala or Val); Xaa at res.58 = (Asn, Lys, Ala or Glu); Xaa at res.59 = (Pro or Ser); Xaa at res.60 = (Glu, Asp, or Gly); Xaa at res.61 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro or Asp); Xaa at res.64 = (Lys or Leu); Xaa at res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val); Xaa at res.70 = (Thr or Ala); Xaa at res.71 = (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or Val); Xaa at res.73 = (Asn, Ser or Asp); Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr or Val); Xaa at res.76 = (Ser or Ala); Xaa at res.77 = (Val or Met); Xaa at res.79 = (Tyr or Phe); Xaa at res.80 = (Phe, Tyr or Leu); Xaa at res.81 = (Asp or Asn); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn or Tyr); Xaa at res.84 = (Ser, Asn, Asp or Glu); Xaa at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile or Val); Xaa at res.89 = (Lys or Arg); Xaa at res.90 = (Lys, Asn, Gln or His); Xaa at res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln or Glu); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 = (Val, Thr or Ala); Xaa at res.97 = (Arg, Lys, Val, Asp or Glu); Xaa at res.98 = (Ala, Gly or Glu); and Xaa at res.102 = (His or Arg).

Similarly, Generic Sequence 5 (Seq. ID No. 30) and Generic Sequence 6 (Seq. ID No. 31) accommodate the homologies shared among all the morphogen protein family members identified in Table II. Specifically, Generic Sequences 5 and 6 are composite amino acid sequences of human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (from mouse, Seq. ID No. 14), human BMP3 (Seq. ID No. 26), human BMP5 (Seq. ID No. 27), human BMP6 (Seq. ID No. 28) and 60(A) (from Drosophila, Seq. ID Nos. 24-25). The generic sequences include both the amino acid identity shared by these sequences in the C-terminal domain, defined by the six and seven-cysteine skeletons (Generic Sequences 5 and 6, respectively), as well as alternative residues for the variable positions within the sequence. As for Generic Sequences 3 and 4, Generic Sequences 5 and 6 allow for an additional cysteine at position 41 (Generic Sequence 5) or position 46 (Generic Sequence 6), providing an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and containing certain critical amino acids which influence the tertiary structure of the proteins.

<u>Generic Sequence 5</u>

Leu Xaa Xaa Xaa Phe
1 5

Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
10

Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
15 20
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
25 30
Xaa Pro Xaa Xaa Xaa Xaa Xaa
35
Xaa Xaa Xaa Asn His Ala Xaa Xaa
40 45
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
55 60
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
65
Xaa Xaa Xaa Leu Xaa Xaa Xaa
70 75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
80
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
85 90
Xaa Cys Xaa Cys Xaa
95

wherein each Xaa is independently selected from a group of one or more specified amino acids defined as follows: "Res." means "residue" and Xaa at res.2 = (Tyr or Lys); Xaa at res.3 = Val or Ile); Xaa at res.4 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser, Lys or Ala); Xaa at res.7 = (Asp, Glu or Lys); Xaa at res.8

= (Leu, Val or Ile); Xaa at res.11 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.12 = (Asp, Arg, Asn or Glu); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.16 (Ala or Ser); Xaa at res.18 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.19 = (Gly or Ser); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at res.28 = (Glu, Lys, Asp, Gln or Ala); Xaa at res.30 = (Ala, Ser, Pro, Gln or Asn); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu, Val or Met); Xaa at res.34 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.35 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn, Ser or Lys); Xaa at res.39 = (Ala, Ser, Gly or Pro); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile, Val or Thr); Xaa at res.45 = (Val, Leu or Ile); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.48 = (Leu or Ile); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His, Asn or Arg); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala, Val or Leu); Xaa at res.53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res.54 = (Pro, Ser or Val); Xaa at res.55 = (Glu, Asp, Asn, Gly, Val or Lys); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys, Leu or Glu); Xaa at res.60 = (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr, Ala or Glu); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser, Asp or Gly); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr, Val or Leu); Xaa at res.71 = (Ser, Ala or Pro); Xaa at res.72 = (Val, Met

or Ile); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr, Leu or His); Xaa at res.76 = (Asp, Asn or Leu); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.79 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile, Val or Asn); Xaa at res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln, His or Val); Xaa at res.86 = (Tyr or His); Xaa at res.87 = (Arg, Gln, Glu or Pro); Xaa at res.88 = (Asn, Glu or Asp); Xaa at res.90 = (Val, Thr, Ala or Ile); Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at res.93 = (Ala, Gly, Glu or Ser); Xaa at res.95 = (Gly or Ala) and Xaa at res.97 = (His or Arg).

Generic Sequence 6

```
Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe
 1               5                  10
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                15
Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
 20                  25
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
        30                  35
Xaa Pro Xaa Xaa Xaa Xaa Xaa
                40
Xaa Xaa Xaa Asn His Ala Xaa Xaa
        45                  50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                55
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
    60                  65
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
            70
```

Xaa Xaa Xaa Leu Xaa Xaa Xaa
75 80
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
85
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
90 95
Xaa Cys Xaa Cys Xaa
100

wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the following: "Res." means "residue" and Xaa at res.2 = (Lys, Arg, Ala or Gln); Xaa at res.3 = (Lys, Arg or Met); Xaa at res.4 = (His, Arg or Gln); Xaa at res.5 = (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr); Xaa at res.7 = (Tyr or Lys); Xaa at res.8 = (Val or Ile); Xaa at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg, Gln, Ser, Lys or Ala); Xaa at res.12 = (Asp, Glu, or Lys); Xaa at res.13 = (Leu, Val or Ile); Xaa at res.16 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.17 = (Asp, Arg, Asn or Glu); Xaa at res.19 = (Ile or Val); Xaa at res.20 = (Ile or Val); Xaa at res.21 = (Ala or Ser); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.24 = (Gly or Ser); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Gln, Leu, or Gly); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at res.33 = Glu, Lys, Asp, Gln or Ala); Xaa at res.35 = (Ala, Ser, Pro, Gln or Asn); Xaa at res.36 = (Phe, Leu or Tyr); Xaa at res.38 = (Leu, Val or Met); Xaa at res.39 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.40 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.41 = (Tyr, Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at res.43 = (Asn, Ser or Lys); Xaa at res.44 = (Ala, Ser, Gly or Pro); Xaa at res.45 = (Thr,

Leu or Ser); Xaa at res.49 = (Ile, Val or Thr); Xaa at res.50 = (Val, Leu or Ile); Xaa at res.51 = (Gln or Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.53 = (Leu or Ile); Xaa at res.54 = (Val or Met); Xaa at res.55 = (His, Asn or Arg); Xaa at res.56 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn, Ala, Val or Leu); Xaa at res.58 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res.59 = (Pro, Ser or Val); Xaa at res.60 = (Glu, Asp, Gly, Val or Lys); Xaa at res.61 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His); Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro or Asp); Xaa at res.64 = (Lys, Leu or Glu); Xaa at res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val); Xaa at res.70 = (Thr, Ala or Glu); Xaa at res.71 = (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or Val); Xaa at res.73 = (Asn, Ser, Asp or Gly); Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr, Val or Leu); Xaa at res.76 = (Ser, Ala or Pro); Xaa at res.77 = (Val, Met or Ile); Xaa at res.79 = (Tyr or Phe); Xaa at res.80 = (Phe, Tyr, Leu or His); Xaa at res.81 = (Asp, Asn or Leu); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.84 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile, Val or Asn); Xaa at res.89 = (Lys or Arg); Xaa at res.90 = (Lys, Asn, Gln, His or Val); Xaa at res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln, Glu or Pro); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 = (Val, Thr, Ala or Ile); Xaa at res.97 = (Arg, Lys, Val, Asp or Glu); Xaa at res.98 = (Ala, Gly, Glu or Ser); Xaa at res.100 = (Gly or Ala); and Xaa at res.102 = (His or Arg).

Particularly useful sequences for use as morphogens in this invention include the C-terminal domains, e.g., the C-terminal 96-102 amino acid residues of Vgl, Vgr-1, DPP, OP-1, OP-2, CBMP-2A, CBMP-2B, GDF-1 (see Table II, below, and Seq. ID Nos. 5-14), as well as proteins comprising the C-terminal domains of 60A, BMP3, BMP5 and BMP6 (see Seq. ID Nos. 24-28), all of which include at least the conserved six or seven cysteine skeleton. In addition, biosynthetic constructs designed from the generic sequences, such as COP-1, 3-5, 7, 16, disclosed in U.S. Pat. No. 5,011,691, also are useful. Other sequences include the inhibins/activin proteins (see, for example, U.S. Pat. Nos. 4,968,590 and 5,011,691). Accordingly, other useful sequences are those sharing at least 70% amino acid sequence homology or "similarity", and preferably 80% homology or similarity with any of the sequences above. These are anticipated to include allelic and species variants and mutants, and biosynthetic muteins, as well as novel members of this morphogenic family of proteins. Particularly envisioned in the family of related proteins are those proteins exhibiting morphogenic activity and wherein the amino acid changes from the preferred sequences include conservative changes, e.g., those as defined by Dayoff et al., Atlas of Protein Sequence and Structure; vol. 5, Suppl. 3, pp. 345-362, (M.O. Dayoff, ed., Nat'l BioMed. Research Fdn., Washington, D.C. 1979). As used herein, potentially useful sequences are aligned with a known morphogen sequence using the method of Needleman et al. ((1970) J.Mol.Biol. 48:443-453) and identities calculated by the Align program (DNAstar, Inc.). "Homology" or "similarity" as used herein includes allowed conservative changes as defined by Dayoff et al.

The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the Drosophila 60A protein. Accordingly, in another preferred aspect of the invention, useful morphogens include active proteins comprising species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX", which accommodates the homologies between the various identified species of OP1 and OP2 (Seq. ID No. 29).

The morphogens useful in the methods, composition and devices of this invention include proteins comprising any of the polypeptide chains described above, whether isolated from naturally-occurring sources, or produced by recombinant DNA or other synthetic techniques, and includes allelic and species variants of these proteins, naturally-occurring or biosynthetic mutants thereof, as well as various truncated and fusion constructs. Deletion or addition mutants also are envisioned to be active, including those which may alter the conserved C-terminal cysteine skeleton, provided that the alteration does not functionally disrupt the relationship of these cysteines in the folded structure. Accordingly, such active forms are considered the equivalent of the specifically described constructs disclosed herein.

The proteins may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology, and active truncated or mutated forms of native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

The morphogenic proteins can be expressed from intact or truncated cDNA or from synthetic DNAs in procaryotic or eucaryotic host cells, and purified, cleaved, refolded, and dimerized to form morphogenically active compositions. Currently preferred host cells include E. coli or mammalian cells, such as CHO, COS or BSC cells. A detailed description of the morphogens useful in the methods, compositions and devices of this invention is disclosed in copending US patent application Serial Nos. 752,764, filed August 30, 1991, and 667,274, filed March 11, 1991, the disclosure of which are incorporated herein by reference.

Thus, in view of this disclosure, skilled genetic engineers can isolate genes from cDNA or genomic libraries of various different species which encode appropriate amino acid sequences, or construct DNAs from oligonucleotides, and then can express them in various types of host cells, including both procaryotes and eucaryotes, to produce large quantities of active proteins capable of protecting tissues and organs from immune cell-mediated tissue destruction, including substantially inhibiting such damage and/or regenerating the damaged tissue in a variety of mammals, including humans.

The foregoing and other objects, features and advantages of the present invention will be made more apparent from the following detailed description of the invention.

**Brief Description of the Drawings**

FIG 1 shows the cardioprotective effects of morphogen (hOP1) in a rat myocardial ischemia-reperfusion model, as evidenced by the smaller loss of myocardial creatine kinase in hOP1-treated rats;

FIG 2 shows the effects of 20 μg of morphogen (hOP1 given 24 hours prior to isolation of rat heart on endothelial-dependent vasorelaxation to acetycholine following induced ischemia-reperfusion injury;

FIG 3 shows the effect of morphogen (hOP1) on neutrophil adherence to $LTB_4$-stimulated mesenteric artery endothelium in neutrophil-activated rats;

FIG 4 (A and B) are schematic representations of morphogen inhibition of early mononuclear phagocytic multinuclearization _in vivo_;

FIG 5 graphs the effect of a morphogen (e.g., OP-1) and a placebo control on mucositic lesion formation; and

FIG 6 (A-D) graphs the effects of a morphogen (eg., OP-1, Figs. 6A and 6C) and TGF-β (Fig. 6B and 6D) on collagen (6A and 6B) and hyaluronic acid (6C and 6D) production in primary fibroblast cultures.

## Detailed Description of the Invention

It now has been surprisingly discovered that the morphogens defined herein are effective agents in alleviating the tissue destructive effects associated with the body's inflammatory response to tissue injury. In particular, as disclosed herein, the morphogens are capable of alleviating the necrotic tissue effects associated with the ensuing inflammatory responses that occur following an initial tissue injury.

When tissue injury occurs, whether caused by bacteria, trauma, chemicals, heat, or any other phenomenon, the body's inflammatory response is stimulated. In response to signals released from the damaged cells (e.g., cytokines), extravascularization of immune effector cells is induced. Under ordinary circumstances these invading immune effector cells kill the infectious agent and/or infected or damaged cells (through the release of killing substances such as superoxides, perforins, and other antimicrobial agents stored in granules), remove the dead tissues and organisms (through phagocytosis), release various biological response modifiers that promote rapid healing and covering of the wound (quite often resulting in the formation of fibrotic scar tissue), and then, after the area is successfully healed, exit from the site of the initial insult. Once the site is perceived to be normal, the local release of inflammatory cytokines ceases and the display of adhesion molecules on the vessel endothelium returns to basal levels. In some cases, however, the zeal of these interacting signals and cellular systems, which are designed to capture and contain very rapidly multiplying infectious agents, act to the detriment of

the body, killing additional, otherwise healthy, surrounding tissue. This additional unnecessary tissue death further compromises organ function and sometimes results in death of the individual. In addition, the resulting scar tissue that often forms can interfere with normal tissue function as occurs, for example, in idiopathic pulmonary fibrosis, IBD and organ cirrhosis.

The vascular endothelium constitutes the first barrier between circulating immune effector cells and extravascular tissues. Extravasation of these circulating cells requires that they bind to the vascular endothelial cells, cross the basement membrane, and enter insulted tissues e.g, by phagocytosis or protease-mediated extracellular matrix degradation. Without being limited to a particular theory, it is believed that the morphogens of this invention may modulate the inflammatory response in part by modulating the attachment of immune effector cells to the luminal side of the endothelium of blood vessels at or near sites of tissue damage and/or inflammatory lesions. Because the method reduces or prevents the attachment of immune effector cells at these sites, it also prevents the subsequent release of tissue destructive agents by these same immune effector cells at sites of tissue damage and/or inflammatory lesions. Because attachment of immune effector cells to the endothelium must precede their extravascularization, the method also prevents the initial or continued entry of these cells into extravascular sites of tissue destruction or ongoing inflammatory lesions. Therefore, the invention not only relates to a method to reduce or prevent the immune cell-mediated cellular destruction at extravascular sites of recent tissue destruction, but

also relates to a method to prevent or reduce the continued entry of immune effector cells into extravascular sites of ongoing inflammatory cascades. As will be appreciated by those skilled in the art, the morphogens of this invention also may be contemplated in mechanisms for disrupting the functional interaction of immune effector cells with endothelium where the adhesion molecules are induced by means other than in response to tissue injury.

One source of tissue injury is induced by cell exposure to toxic oxygen concentrations, such as ischemic-reperfusion tissue injury (oxygen deprivation), and following hyperoxia injury (lethally high oxygen concentrations). Accordingly, the process of the present invention provides a method for alleviating the tissue damage induced by ischemic-reperfusion injury or hyperoxia-induced injury comprising the step of administering to the afflicted individual a therapeutic amount of a morphogen prior to, during, or after damage to the affected tissue. Where the toxic oxygen concentrations may be deliberately induced, as by a surgical or clinical procedure, the morphogen preferably is administered prior to induction.

In addition, the morphogens described herein, in contrast to fibrogenic growth factors such as TGF-$\beta$, stimulate tissue morphogenesis and do not stimulate fibrosis or scar tissue formation (see Example 9, below.) Accordingly, in addition to inhibiting the tissue destructive effects associated with the inflammatory response, the morphogens further enhance the viability of damaged tissue and/or organs by stimulating the regeneration of the damaged tissue and preventing fibrogenesis.

The morphogens described herein also can inhibit epithelial cell proliferation (see Example 10, below.) This activity of the morphogens also may be particularly useful in the treatment of psoriasis and other inflammatory diseases that involve epithelial cell populations.

Provided below are detailed descriptions of suitable morphogens useful in the methods and compositions of this invention, as well as methods for their administration and application, and numerous, nonlimiting examples which 1) illustrate the suitability of the morphogens and morphogen-stimulating agents described herein as therapeutic agents for protecting tissue from the tissue destructive effects associated with the body's inflammatory response; and 2) provide assays with which to test candidate morphogens and morphogen-stimulating agents for their efficacy.

## I. Useful Morphogens

As defined herein a protein is morphogenic if it is capable of inducing the developmental cascade of cellular and molecular events that culminate in the formation of new, organ-specific tissue and comprises at least the conserved C-terminal six cysteine skeleton or its functional equivalent (see supra). Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting

the growth and maintenance of differentiated cells, including the "redifferentiation" of transformed cells. Details of how the morphogens useful in the method of this invention first were identified, as well as a description on how to make, use and test them for morphogenic activity are disclosed in USSN 667,274, filed March 11, 1991 and USSN 752,764, filed August 30, 1991, the disclosures of which are hereinabove incorporated by reference. As disclosed therein, the morphogens may be purified from naturally-sourced material or recombinantly produced from procaryotic or eucaryotic host cells, using the genetic sequences disclosed therein. Alternatively, novel morphogenic sequences may be identified following the procedures disclosed therein.

Particularly useful proteins include those which comprise the naturally derived sequences disclosed in Table II. Other useful sequences include biosynthetic constructs such as those disclosed in U.S. Pat. 5,011,691, the disclosure of which is incorporated herein by reference (e.g., COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16).

Accordingly, the morphogens useful in the methods and compositions of this invention also may be described by morphogenically active proteins having amino acid sequences sharing 70% or, preferably, 80% homology (similarity) with any of the sequences described above, where "homology" is as defined herein above.

The morphogens useful in the method of this invention also can be described by any of the 6 generic sequences described herein (Generic Sequences 1, 2, 3, 4, 5 and 6). Generic sequences 1 and 2 also may include, at their N-terminus, the sequence

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)
1 5

Table II, set forth below, compares the amino acid sequences of the active regions of native proteins that have been identified as morphogens, including human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-23), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), BMP3 (Seq. ID No. 26), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), GDF-1 (from mouse, Seq. ID Nos. 14, 32 and 33), 60A protein (from Drosophila, Seq. ID Nos. 24 and 25), BMP5 (Seq. ID No. 27) and BMP6 (Seq. ID No. 28). The sequences are aligned essentially following the method of Needleman et al. (1970) J. Mol. Biol., 48:443-453, calculated using the Align Program (DNAstar, Inc.) In the table, three dots indicates that the amino acid in that position is the same as the amino acid in hOP-1. Three dashes indicates that no amino acid is present in that position, and are included for purposes of illustrating homologies. For example, amino acid residue 60 of CBMP-2A and CBMP-2B is "missing". Of course, both these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with CBMP-2A then comprising Lys and Ile, whereas CBMP-2B comprises Ser and Ile.

TABLE II

| hOP-1 | Cys | Lys | Lys | His | Glu | Leu | Tyr | Val |
|---|---|---|---|---|---|---|---|---|
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | Arg | Arg | ... | ... | ... | ... | ... |
| mOP-2 | ... | Arg | Arg | ... | ... | ... | ... | ... |
| DPP | ... | Arg | Arg | ... | Ser | ... | ... | ... |
| Vgl | ... | ... | Lys | Arg | His | ... | ... | ... |
| Vgr-1 | ... | ... | ... | ... | Gly | ... | ... | ... |
| CBMP-2A | ... | ... | Arg | ... | Pro | ... | ... | ... |
| CBMP-2B | ... | Arg | Arg | ... | Ser | ... | ... | ... |
| BMP3 | ... | Ala | Arg | Arg | Tyr | ... | Lys | ... |
| GDF-1 | ... | Arg | Ala | Arg | Arg | ... | ... | ... |
| 60A | ... | Gln | Met | Glu | Thr | ... | ... | ... |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | Arg | ... | ... | ... | ... | ... | ... |
| | 1 | | | | 5 | | | |

| hOP-1 | Ser | Phe | Arg | Asp | Leu | Gly | Trp | Gln | Asp |
|---|---|---|---|---|---|---|---|---|---|
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | Gln | ... | ... | ... | ... | Leu | ... |
| mOP-2 | Ser | ... | ... | ... | ... | ... | ... | Leu | ... |
| DPP | Asp | ... | Ser | ... | Val | ... | ... | Asp | ... |
| Vgl | Glu | ... | Lys | ... | Val | ... | ... | ... | Asn |
| Vgr-1 | ... | ... | Gln | ... | Val | ... | ... | ... | ... |
| CBMP-2A | Asp | ... | Ser | ... | Val | ... | ... | Asn | ... |
| CBMP-2B | Asp | ... | Ser | ... | Val | ... | ... | Asn | ... |
| BMP3 | Asp | ... | Ala | ... | Ile | ... | ... | Ser | Glu |
| GDF-1 | ... | ... | ... | Glu | Val | ... | ... | His | Arg |
| 60A | Asp | ... | Lys | ... | ... | ... | ... | His | ... |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | Gln | ... | ... | ... | ... | ... | ... |
| | | 10 | | | | | 15 | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| hOP-1 | Trp | Ile | Ile | Ala | Pro | Glu | Gly | Tyr | Ala |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | Val | ... | ... | ... | Gln | ... | ... | Ser |
| mOP-2 | ... | Val | ... | ... | ... | Gln | ... | ... | Ser |
| DPP | ... | ... | Val | ... | ... | Leu | ... | ... | Asp |
| Vgl | ... | Val | ... | ... | ... | Gln | ... | ... | Met |
| Vgr-1 | ... | ... | ... | ... | ... | Lys | ... | ... | ... |
| CBMP-2A | ... | ... | Val | ... | ... | Pro | ... | ... | His |
| CBMP-2B | ... | ... | Val | ... | ... | Pro | ... | ... | Gln |
| BMP3 | ... | ... | ... | Ser | ... | Lys | Ser | Phe | Asp |
| GDF-1 | ... | Val | ... | ... | ... | Arg | ... | Phe | Leu |
| 60A | ... | ... | ... | ... | ... | ... | ... | ... | Gly |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | ... | ... | ... | Lys | ... | ... | ... |
| | | | 20 | | | | | 25 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| hOP-1 | Ala | Tyr | Tyr | Cys | Glu | Gly | Glu | Cys | Ala |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | ... | ... | ... | ... | ... | ... | Ser |
| mOP-2 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| DPP | ... | ... | ... | ... | His | ... | Lys | ... | Pro |
| Vgl | ... | Asn | ... | ... | Tyr | ... | ... | ... | Pro |
| Vgr-1 | ... | Asn | ... | ... | Asp | ... | ... | ... | Ser |
| CBMP-2A | ... | Phe | ... | ... | His | ... | Glu | ... | Pro |
| CBMP-2B | ... | Phe | ... | ... | His | ... | Asp | ... | Pro |
| BMP3 | ... | ... | ... | ... | Ser | ... | Ala | ... | Gln |
| GDF-1 | ... | Asn | ... | ... | Gln | ... | Gln | ... | ... |
| 60A | ... | Phe | ... | ... | Ser | ... | ... | ... | Asn |
| BMP5 | ... | Phe | ... | ... | Asp | ... | ... | ... | Ser |
| BMP6 | ... | Asn | ... | ... | Asp | ... | ... | ... | Ser |
| | | | | 30 | | | | | 35 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| hOP-1 | Phe | Pro | Leu | Asn | Ser | Tyr | Met | Asn | Ala |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| hOP-2 | ... | ... | ... | Asp | ... | Cys | ... | ... | ... |
| mOP-2 | ... | ... | ... | Asp | ... | Cys | ... | ... | ... |
| DPP | ... | ... | ... | Ala | Asp | His | Phe | ... | Ser |
| Vgl | Tyr | ... | ... | Thr | Glu | Ile | Leu | ... | Gly |
| Vgr-1 | ... | ... | ... | ... | Ala | His | ... | ... | ... |
| CBMP-2A | ... | ... | ... | Ala | Asp | His | Leu | ... | Ser |
| CBMP-2B | ... | ... | ... | Ala | Asp | His | Leu | ... | Ser |
| GDF-1 | Leu | ... | Val | Ala | Leu | Ser | Gly | Ser** | ... |
| BMP3 | ... | ... | Met | Pro | Lys | Ser | Leu | Lys | Pro |
| 60A | ... | ... | ... | ... | Ala | His | ... | ... | ... |
| BMP5 | ... | ... | ... | ... | Ala | His | Met | ... | ... |
| BMP6 | ... | ... | ... | ... | Ala | His | Met | ... | ... |
| | | | | | 40 | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| hOP-1 | Thr | Asn | His | Ala | Ile | Val | Gln | Thr | Leu |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | ... | ... | ... | Leu | ... | Ser | ... |
| mOP-2 | ... | ... | ... | ... | ... | Leu | ... | Ser | ... |
| DPP | ... | ... | ... | ... | Val | ... | ... | ... | ... |
| Vgl | Ser | ... | ... | ... | ... | Leu | ... | ... | ... |
| Vgr-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| CBMP-2A | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| CBMP-2B | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP3 | Ser | ... | ... | ... | Thr | Ile | ... | Ser | Ile |
| GDF-1 | Leu | ... | ... | ... | Val | Leu | Arg | Ala | ... |
| 60A | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| | 45 | | | | | 50 | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| hOP-1 | Val | His | Phe | Ile | Asn | Pro | Glu | Thr | Val |
| mOP-1 | ... | ... | ... | ... | ... | ... | Asp | ... | ... |
| hOP-2 | ... | His | Leu | Met | Lys | ... | Asn | Ala | ... |
| mOP-2 | ... | His | Leu | Met | Lys | ... | Asp | Val | ... |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| DPP | ... | Asn | Asn | Asn | ... | ... | Gly | Lys | ... |
| Vgl | ... | ... | Ser | ... | Glu | ... | ... | Asp | Ile |
| Vgr-1 | ... | ... | Val | Met | ... | ... | ... | Tyr | ... |
| CBMP-2A | ... | Asn | Ser | Val | ... | Ser | --- | Lys | Ile |
| CBMP-2B | ... | Asn | Ser | Val | ... | Ser | --- | Ser | Ile |
| BMP3 | ... | Arg | Ala** | Gly | Val | Val | Pro | Gly | Ile |
| GDF-1 | Met | ... | Ala | Ala | Ala | ... | Gly | Ala | Ala |
| 60A | ... | ... | Leu | Leu | Glu | ... | Lys | Lys | ... |
| BMP5 | ... | ... | Leu | Met | Phe | ... | Asp | His | ... |
| BMP6 | ... | ... | Leu | Met | ... | ... | ... | Tyr | ... |
| | | 55 | | | | | 60 | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| hOP-1 | Pro | Lys | Pro | Cys | Cys | Ala | Pro | Thr | Gln |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | Ala | ... | ... | ... | ... | ... | Lys |
| mOP-2 | ... | ... | Ala | ... | ... | ... | ... | ... | Lys |
| DPP | ... | ... | Ala | ... | ... | Val | ... | ... | ... |
| Vgl | ... | Leu | ... | ... | ... | Val | ... | ... | Lys |
| Vgr-1 | ... | ... | ... | ... | ... | ... | ... | ... | Lys |
| CBMP-2A | ... | ... | Ala | ... | ... | Val | ... | ... | Glu |
| CBMP-2B | ... | ... | Ala | ... | ... | Val | ... | ... | Glu |
| BMP3 | ... | Glu | ... | ... | ... | Val | ... | Glu | Lys |
| GDF-1 | Asp | Leu | ... | ... | ... | Val | ... | Ala | Arg |
| 60A | ... | ... | ... | ... | ... | ... | ... | ... | Arg |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | Lys |
| BMP6 | ... | ... | ... | ... | ... | ... | ... | ... | Lys |
| | | | 65 | | | | | 70 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| hOP-1 | Leu | Asn | Ala | Ile | Ser | Val | Leu | Tyr | Phe |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | Ser | ... | Thr | ... | ... | ... | ... | Tyr |
| mOP-2 | ... | Ser | ... | Thr | ... | ... | ... | ... | Tyr |
| Vgl | Met | Ser | Pro | ... | ... | Met | ... | Phe | Tyr |
| Vgr-1 | Val | ... | ... | ... | ... | ... | ... | ... | ... |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| DPP | ... | Asp | Ser | Val | Ala | Met | ... | ... | Leu |
| CBMP-2A | ... | Ser | ... | ... | ... | Met | ... | ... | Leu |
| CBMP-2B | ... | Ser | ... | ... | ... | Met | ... | ... | Leu |
| BMP3 | Met | Ser | Ser | Leu | ... | Ile | ... | Phe | Tyr |
| GDF-1 | ... | Ser | Pro | ... | ... | ... | ... | Phe | ... |
| 60A | ... | Gly | ... | Leu | Pro | ... | ... | ... | His |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| | | | | 75 | | | | | 80 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| hOP-1 | Asp | Asp | Ser | Ser | Asn | Val | Ile | Leu | Lys |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | Ser | ... | Asn | ... | ... | ... | ... | Arg |
| mOP-2 | ... | Ser | ... | Asn | ... | ... | ... | ... | Arg |
| DPP | Asn | ... | Gln | ... | Thr | ... | Val | ... | ... |
| Vgl | ... | Asn | Asn | Asp | ... | ... | Val | ... | Arg |
| Vgr-1 | ... | ... | Asn | ... | ... | ... | ... | ... | ... |
| CBMP-2A | ... | Glu | Asn | Glu | Lys | ... | Val | ... | ... |
| CBMP-2B | ... | Glu | Tyr | Asp | Lys | ... | Val | ... | ... |
| BMP3 | ... | Glu | Asn | Lys | ... | ... | Val | ... | ... |
| GDF-1 | ... | Asn | ... | Asp | ... | ... | Val | ... | Arg |
| 60A | Leu | Asn | Asp | Glu | ... | ... | Asn | ... | ... |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | Asn | ... | ... | ... | ... | ... | ... |
| | | | | | 85 | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| hOP-1 | Lys | Tyr | Arg | Asn | Met | Val | Val | Arg |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | His | ... | ... | ... | ... | ... | Lys |
| mOP-2 | ... | His | ... | ... | ... | ... | ... | Lys |
| DPP | Asn | ... | Gln | Glu | ... | Thr | ... | Val |
| Vgl | His | ... | Glu | ... | ... | Ala | ... | Asp |
| Vgr-1 | ... | ... | ... | ... | ... | ... | ... | ... |
| CBMP-2A | Asn | ... | Gln | Asp | ... | ... | ... | Glu |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| CBMP-2B | Asn | ... | Gln | Glu | ... | ... | ... | Glu |
| BMP3 | Val | ... | Pro | ... | ... | Thr | ... | Glu |
| GDF-1 | Gln | ... | Glu | Asp | ... | ... | ... | Asp |
| 60A | ... | ... | ... | ... | ... | Ile | ... | Lys |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | ... | Trp | ... | ... | ... | ... |
| | 90 | | | | | 95 | | |

| | | | | | |
|---|---|---|---|---|---|
| hOP-1 | Ala | Cys | Gly | Cys | His |
| mOP-1 | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | ... | ... | ... |
| mOP-2 | ... | ... | ... | ... | ... |
| DPP | Gly | ... | ... | ... | Arg |
| Vgl | Glu | ... | ... | ... | Arg |
| Vgr-1 | ... | ... | ... | ... | ... |
| CBMP-2A | Gly | ... | ... | ... | Arg |
| CBMP-2B | Gly | ... | ... | ... | Arg |
| BMP3 | Ser | ... | Ala | ... | Arg |
| GDF-1 | Glu | ... | ... | ... | Arg |
| 60A | Ser | ... | ... | ... | ... |
| BMP5 | Ser | ... | ... | ... | ... |
| BMP6 | ... | ... | ... | ... | ... |
| | | | 100 | | |

**Between residues 56 and 57 of BMP3 is a Val residue; between residues 43 and 44 of GDF-1 lies the amino acid sequence Gly-Gly-Pro-Pro.

As is apparent from the foregoing amino acid sequence comparisons, significant amino acid changes can be made within the generic sequences while retaining the morphogenic activity. For example, while the GDF-1 protein sequence depicted in Table II shares only about 50% amino acid identity with the hOP1

sequence described therein, the GDF-1 sequence shares greater than 70% amino acid sequence homology (or "similarity") with the hOP1 sequence, where "homology" or "similarity" includes allowed conservative amino acid changes within the sequence as defined by Dayoff, et al., <u>Atlas of Protein Sequence and Structure</u> vol.5, supp.3, pp.345-362, (M.O. Dayoff, ed., Nat'l BioMed. Res. Fd'n, Washington D.C. 1979.)

The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the Drosophila 60A protein. Accordingly, in still another preferred aspect, the invention includes morphogens comprising species of polypeptide chains having the generic amino acid sequence referred to herein as "OPX", which defines the seven cysteine skeleton and accommodates the identities between the various identified mouse and human OP1 and OP2 proteins. OPX is presented in Seq. ID No. 29. As described therein, each Xaa at a given position independently is selected from the residues occurring at the corresponding position in the C-terminal sequence of mouse or human OP1 or OP2 (see Seq. ID Nos. 5-8 and/or Seq. ID Nos. 16-23).

## II. Formulations and Methods for Administering Therapeutic Agents

The morphogens may be provided to an individual by any suitable means, preferably directly (e.g., locally, as by injection or topical administration to a tissue locus) or systemically (e.g., parenterally or orally). Where the morphogen is to be provided parenterally, such as by intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intraventricular, intracranial, intracapsular, intraspinal, intracisternal, intraperitoneal, buccal, rectal, vaginal, intranasal or by aerosol administration, the morphogen preferably comprises part of an aqueous solution. The solution is physiologically acceptable so that in addition to delivery of the desired morphogen to the patient, the solution does not otherwise adversely affect the patient's electrolyte and volume balance. The aqueous medium for the morphogen thus may comprise normal physiologic saline (9.85% NaCl, 0.15M), pH 7-7.4. The aqueous solution containing the morphogen can be made, for example, by dissolving the protein in 50% ethanol containing acetonitrile in 0.1% trifluoroacetic acid (TFA) or 0.1% HCl, or equivalent solvents. One volume of the resultant solution then is added, for example, to ten volumes of phosphate buffered saline (PBS), which further may include 0.1-0.2% human serum albumin (HSA). The resultant solution preferably is vortexed extensively. If desired, a given morphogen may be made more soluble by association with a suitable molecule. For example, association of the mature dimer with the pro domain of the morphogen keeps the morphogen soluble in physiological buffers. In fact, the endogenous protein is thought to be transported in this form.

Another molecule capable of enhancing solubility and particularly useful for oral administrations, is casein. For example, addition of 0.2% casein increases solubility of the mature active form of OP-1 by 80%. Other components found in milk and/or various serum proteins also may be useful.

Useful solutions for parenteral administration may be prepared by any of the methods well known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences (Gennaro, A., ed.), Mack Pub., 1990. Formulations may include, for example, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and the like. Formulations for direct administration, in particular, may include glycerol and other compositions of high viscosity to help maintain the morphogen at the desired locus. Biocompatible, preferably bioresorbable, polymers, including, for example, hyaluronic acid, collagen, tricalcium phosphate, polybutyrate, lactide and glycolide polymers, and lactide/glycolide copolymers, may be useful excipients to control the release of the morphogen *in vivo*. Other potentially useful parenteral delivery systems for these morphogens include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation administration contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally.

Formulations for parenteral administration may also include glycocholate for buccal administration, methoxysalicylate for rectal administration, or cutric acid for vaginal administration.

Suppositories for rectal administration also may be prepared by mixing the morphogen or morphogen-stimulating agent with a non-irritating excipient such as cocoa butter or other compositions which are solid at room temperature and liquid at body temperatures.

Formulations for topical administration to the skin surface may be prepared by dispersing the morphogen or morphogen-stimulating agent with a dermally acceptable carrier such as a lotion, cream, ointment or soap. Particularly useful are carriers capable of forming a film or layer over the skin to localize application and inhibit removal. For topical administration to internal tissue surfaces, the morphogen may be dispersed in a liquid tissue adhesive or other substance known to enhance adsorption to a tissue surface. For example, hydroxypropylcellulose or fibrinogen/thrombin solutions may be used to advantage. Alternatively, tissue-coating solutions, such as pectin-containing formulations, may be used.

Alternatively, the morphogens described herein may be administered orally. Oral administration of proteins as therapeutics generally is not practiced as most proteins are readily degraded by digestive enzymes and acids in the mammalian digestive system before they can be absorbed into the bloodstream. However, the morphogens described herein typically are acid stable and protease-resistant (see, for example, U.S. Pat.No. 4,968,590.) In addition, at least one morphogen, OP-1,

has been identified in mammary gland extract, colostrum and 57-day milk. Moreover, the OP-1 purified from mammary gland extract is morphogenically active. Specifically, this protein induces endochondral bone formation in mammals when implanted subcutaneously in association with a suitable matrix material, using a standard *in vivo* bone assay, such as is disclosed in U.S. Pat.No. 4,968,590. Moreover, the morphogen also is detected in the bloodstream. Finally, soluble form morphogen, e.g., mature morphogen associated with the pro domain, is morphogenically active. These findings indicate that oral and parenteral administration are viable means for administering morphogens to an individual. In addition, while the mature forms of certain morphogens described herein typically are sparingly soluble, the morphogen form found in milk (and mammary gland extract and colostrum) is readily soluble, probably by association of the mature, morphogenically active form with part or all of the pro domain of the intact sequence and/or by association with one or more milk components. Accordingly, the compounds provided herein also may be associated with molecules capable of enhancing their solubility *in vitro* or *in vivo*.

Where the morphogen or morphogen-stimulating agent comprises part of a tissue or organ preservation solution, any commercially available preservation solution may be used to advantage. For example, useful solutions known in the art include Collins solution, Wisconsin solution, Belzer solution, Eurocollins solution and lactated Ringer's solution. Generally, an organ preservation solution usually possesses one or more of the following properties: (a) an osmotic pressure substantially equal to that of the inside of a

mammalian cell,(solutions typically are hyperosmolar and have K+ and/or Mg++ ions present in an amount sufficient to produce an osmotic pressure slightly higher than the inside of a mammalian cell; (b) the solution typically is capable of maintaining substantially normal ATP levels in the cells; and (c) the solution usually allows optimum maintenance of glucose metabolism in the cells. Organ preservation solutions also may contain anticoagulants, energy sources such as glucose, fructose and other sugars, metabolites, heavy metal chelators, glycerol and other materials of high viscosity to enhance survival at low temperatures, free oxygen radical inhibiting agents and a pH indicator. A detailed description of preservation solutions and useful components may be found, for example, in US Patent No. 5,002,965, the disclosure of which is incorporated herein by reference.

The compounds provided herein also may be associated with molecules capable of targeting the morphogen or morphogen-stimulating agent to the desired tissue. For example, an antibody, antibody fragment, or other binding protein that interacts specifically with a surface molecule on cells of the desired tissue, may be used. Useful targeting molecules may be designed, for example, using the single chain binding site technology disclosed, for example, in U.S. Pat. No. 5,091,513.

As described above, the morphogens provided herein share significant sequence homology in the C-terminal active domains. By contrast, the sequences typically diverge significantly in the sequences which define the pro domain. Accordingly, the pro domain is thought to

be morphogen-specific. As described above, it is also known that the various morphogens identified to date are differentially expressed in the different tissues. Accordingly, without being limited to any given theory, it is likely that, under natural conditions in the body, selected morphogens typically act on a given tissue. Accordingly, part or all of the pro domains which have been identified associated with the active form of the morphogen in solution, may serve as targeting molecules for the morphogens described herein. For example, the pro domains may interact specifically with one or more molecules at the target tissue to direct the morphogen associated with the pro domain to that tissue. Accordingly, another useful targeting molecule for targeting morphogen to a tissue of interest is part or all of a morphogen pro domain. For example, part or all of the pro domain of GDF-1 may be used to target a morphogen to nerve tissue. Alternatively, part or all of the pro domain of OP-1 or CBMP2 may be used to target a morphogen to bone tissue, both of which proteins are found naturally associated with bone tissue.

The morphogens described herein are useful for providing neuroprotective effects to alleviate neural pathway damage associated with the body's immune/inflammatory response to an initial injury to nerve tissue. As used herein, a "neural pathway" describes a nerve circuit for the passage of electric signals from a source to a target cell site and includes both the central nervous system (CNS) and peripheral nervous system (PNS). The pathway includes the neurons through which the electric impulse is transported, including groups of interconnecting neurons, the nerve fibers formed by bundled neuronal

axons, and the glial cells surrounding and associated with the neurons. An inflammatory response to nerve tissue injury may follow trauma to nerve tissue, caused, for example, by an autoimmune (including autoantibody) dysfunction, neoplastic lesion, infection, chemical or mechanical trauma, or other disease. An exemplary nerve-related inflammatory disease is multiple sclerosis. Neural pathway damage also can result from a reduction or interruption, e.g., occlusion, of a neural blood supply, as in an embolic stroke, (e.g, ischemia or hypoxia-induced injury), or by other trauma to the nerve or surrounding material. In addition, at least part of the damage associated with a number of primary brain tumors also appears to be immunologically related. Application of the morphogen directly to the cells to be treated, or providing the morphogen to the mammal systemically, for example, intravenously or indirectly by oral administration, may be used to alleviate and/or inhibit the immunologically related response to a neural injury. Alternatively, administration of an agent capable of stimulating morphogen expression and/or secretion in vivo, preferably at the site of injury, also may be used. Where the injury is to be induced, as during surgery or other aggressive clinical treatment, the morphogen or agent may be provided prior to induction of the injury to provide a neuroprotective effect to the nerve tissue at risk.

Where the morphogen is intended for use as a therapeutic to alleviate tissue damage associated with an immune/inflammatory condition of the CNS, an additional problem must be addressed: overcoming the so-called "blood-brain barrier", the brain capillary wall structure that effectively screens out all but

selected categories of molecules present in the blood, preventing their passage into the brain. The blood-brain barrier may be bypassed effectively by direct infusion of the morphogen or morphogen-stimulating agent into the brain. Alternatively, the morphogen or morphogen-stimulating agent may be modified to enhance its transport across the blood-brain barrier. For example, truncated forms of the morphogen or a morphogen-stimulating agent may be most successful. Alternatively, the morphogen or morphogen-stimulating agent may be modified to render it more lipophilic, or it may be conjugated to another molecule which is naturally transported across the barrier, using standard means known to those skilled in the art, as, for example, described in Pardridge, Endocrine Reviews 7:314-330 (1986) and U.S. Pat. No. 4,801,575.

Finally, the morphogens or morphogen-stimulating agents provided herein may be administered alone or in combination with other molecules known to be beneficial in the treatment compositions and methods described herein, including, but not limited to anticoagulants, free oxygen radical inhibiting agents, salicylic acid, vitamin D, and other antiinflammatory agents. Psoriais treatments also may include ultra-violet light treatment, zinc oxide and retinoids.

The compounds provided herein can be formulated into pharmaceutical compositions by admixture with pharmaceutically acceptable nontoxic excipients and carriers. As noted above, such compositions may be prepared for parenteral administration, particularly in

the form of liquid solutions or suspensions; for oral administration, particularly in the form of tablets or capsules; or intranasally, particularly in the form of powders, nasal drops, or aerosols.

The compositions can be formulated for parenteral or oral administration to humans or other mammals in therapeutically effective amounts, e.g., amounts which provide appropriate concentrations for a time sufficient to alleivate the tissue destructive effects associated with the inflammatory response, including protecting tissue in anticipation of tissue damage.

As will be appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition will vary depending upon a number of factors, including the dosage of the drug to be administered, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, and the route of administration. The preferred dosage of drug to be administered also is likely to depend on such variables as the type and extent of progression of the tissue damage, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the compound excipients, and its route of administration. In general terms, the compounds of this invention may be provided in an aqueous physiological buffer solution containing about 0.001% to 10% w/v compound for parenteral administration. Typical dose ranges are from about 10 ng/kg to about 1 g/kg of body weight per day; a preferred dose range is from about 0.1 μg/kg to 100 mg/kg of body weight per day. Optimally, the morphogen dosage given is between 0.1-100 μg of protein per kilogram weight of the patient. No obvious

morphogen induced pathological lesions are induced when mature morphogen (e.g., OP-1, 20 μg) is administered daily to normal growing rats for 21 consecutive days. Moreover, 10 μg systemic injections of morphogen (e.g., OP-1) injected daily for 10 days into normal newborn mice does not produce any gross abnormalities.

In administering morphogens systemically in the methods of the present invention, preferably a large volume loading dose is used at the start of the treatment. The treatment then is continued with a maintenance dose. Further administration then can be determined by monitoring at intervals the levels of the morphogen in the blood.

Where tissue injury is induced deliberately as part of, for example, a surgical procedure, the morphogen preferably is provided just prior to, or concomitant with induction of the trauma. Preferably, the morphogen is administered prophylactically in a surgical setting.

Alternatively, an effective amount of an agent capable of stimulating endogenous morphogen levels may be administered by any of the routes described above. For example, an agent capable of stimulating morphogen production and/or secretion from cells of affected tissue and/or transplant tissue may be provided to a mammal, e.g., by direct administration of the agent to the tissue to be treated. A method for identifying and testing agents capable of modulating the levels of endogenous morphogens in a given tissue is described generally herein in Example 15, and in detail in copending USSN 752,859, filed August 30, 1991, the disclosure of which is incorporated herein by reference. Briefly, candidate compounds can be

identified and tested by incubating the compound *in vitro* with a test tissue or cells thereof, for a time sufficient to allow the compound to affect the production, i.e., the expression and/or secretion, of a morphogen produced by the cells of that tissue.

For purposes of the present invention, the above-described morphogens effective in alleviating tissue damage associated with ischemic-reperfusion injury (or the agents that stimulate them, referred to collectively herein as "therapeutic agent") are administered prior to or during the restoration of oxygen (e.g., restoration of blood flow, reperfusion.) Where treatment is to follow an existing injury, the therapeutic agent preferably is administered as an intravenous infusion provided acutely after the hypoxic or ischemic condition occurs. For example, the therapeutic agent can be administered by intravenous infusion immediately after a cerebral infarction, a myocardial infarction, asphyxia, or a cardiopulmonary arrest. Where ischemia or hypoxia injury is deliberately and/or unavoidably induced as part of, for example, a surgical procedure where circulation to an organ or organ system is deliberately and/or transiently interrupted, e.g., in carotid enterectomy, coronary artery bypass, grafting, organ transplanting, fibrinolytic therapy, etc., the therapeutic agent preferably is provided just prior to, or concomitant with, reduction of oxygen to the tissue. Preferably, the morphogen is administered prophylactically in a surgical setting.

Similarly, where hyperoxia-induced injury already has occurred, the morphogen is administered upon diagnosis. Where hyperoxia injury may be induced as,

for example, during treatment of prematurely newborn babies, or patients suffering from pulmonary diseases such as emphysema, the therapeutic agent preferably is administered prior to administration of oxygen e.g., prophylactically.

## III. Examples

### Example 1. Identification of Morphogen-Expressing Tissue

Determining the tissue distribution of morphogens may be used to identify different morphogens expressed in a given tissue, as well as to identify new, related morphogens. Tissue distribution also may be used to identify useful morphogen-producing tissue for use in screening and identifying candidate morphogen-stimulating agents. The morphogens (or their mRNA transcripts) readily are identified in different tissues using standard methodologies and minor modifications thereof in tissues where expression may be low. For example, protein distribution may be determined using standard Western blot analysis or immunofluorescent techniques, and antibodies specific to the morphogen or morphogens of interest. Similarly, the distribution of morphogen transcripts may be determined using standard Northern hybridization protocols and transcript-specific probes.

Any probe capable of hybridizing specifically to a transcript, and distinguishing the transcript of interest from other, related transcripts may be used. Because the morphogens described herein share such high sequence homology in their active, C-terminal domains,

the tissue distribution of a specific morphogen transcript may best be determined using a probe specific for the pro region of the immature protein and/or the N-terminal region of the mature protein. Another useful sequence is the 3' non-coding region flanking and immediately following the stop codon. These portions of the sequence vary substantially among the morphogens of this invention, and accordingly, are specific for each protein. For example, a particularly useful Vgr-1-specific probe sequence is the PvuII-SacI fragment, a 265 bp fragment encoding both a portion of the untranslated pro region and the N-terminus of the mature sequence (see Lyons et al. (1989) PNAS 86:4554-4558 for a description of the cDNA sequence). Similarly, particularly useful mOP-1-specific probe sequences are the BstX1-BglI fragment, a 0.68 Kb sequence that covers approximately two-thirds of the mOP-1 pro region; a StuI-StuI fragment, a 0.2 Kb sequence immediately upstream of the 7-cysteine domain; and the Earl-Pstl fragment, an 0.3 Kb fragment containing a portion of the 3'untranslated sequence (See Seq. ID No. 18, where the pro region is defined essentially by residues 30-291.) Similar approaches may be used, for example, with hOP-1 (Seq. ID No. 16) or human or mouse OP-2 (Seq. ID Nos. 20 and 22.)

Using these morphogen-specific probes, which may be synthetically engineered or obtained from cloned sequences, morphogen transcripts can be identified in mammalian tissue, using standard methodologies well known to those having ordinary skill in the art. Briefly, total RNA is prepared from various adult murine tissues (e.g., liver, kidney, testis, heart, brain, thymus and stomach) by a standard methodology such as by the method of Chomczyaski et al. ((1987)

primary tissue expression source for Vgr-1, BMP5, BMP4 and BMP3. Lower levels of Vgr-1 also are seen in kidney and heart tissue, while the liver appears to be a secondary expression source for BMP5, and the spleen appears to be a secondary expression source for BMP4. GDF-1 appears to be expressed primarily in brain tissue. To date, OP-2 appears to be expressed primarily in early embryonic tissue. Specifically, northern blots of murine embryos and 6-day post-natal animals shows abundant OP2 expression in 8-day embryos. Expression is reduced significantly in 17-day embryos and is not detected in post-natal animals.

Example 2. Active Morphogens in Body Fluids

OP-1 expression has been identified in saliva (specifically, the rat parotid gland, see Example 1), human blood serum, and various milk forms, including mammary gland extract, colostrum, and 57-day bovine milk. Moreover, and as described in USSN 923,780, the disclosure of which is incorporated herein by reference, the body fluid-extracted protein is morphogenically active. The discovery that the morphogen naturally is present in milk and saliva, together with the known observation that mature, active OP-1 is acid-stable and protease-resistant, indicate that oral administration is a useful route for therapeutic administration of morphogen to a mammal. Oral administration typically is the preferred mode of delivery for extended or prophylactic therapies. In addition, the identification of morphogen in all milk forms, including colostrum, suggests that the protein may play a significant role in tissue development, including skeletal development, of juveniles.

Anal. Biochem 162:156-159) and described below. Poly (A)+ RNA is prepared by using oligo (dT)-cellulose chromatography (e.g., Type 7, from Pharmacia LKB Biotechnology, Inc.). Poly (A)+ RNA (generally 15 μg) from each tissue is fractionated on a 1% agarose/formaldehyde gel and transferred onto a Nytran membrane (Schleicher & Schuell). Following the transfer, the membrane is baked at 80°C and the RNA is cross-linked under UV light (generally 30 seconds at 1 $mW/cm^2$). Prior to hybridization, the appropriate probe is denatured by heating. The hybridization is carried out in a lucite cylinder rotating in a roller bottle apparatus at approximately 1 rev/min for approximately 15 hours at 37°C using a hybridization mix of 40% formamide, 5 x Denhardts, 5 x SSPE, and 0.1% SDS. Following hybridization, the non-specific counts are washed off the filters in 0.1 x SSPE, 0.1% SDS at 50°C.

Examples demonstrating the tissue distribution of various morphogens, including Vgr-1, OP-1, BMP2, BMP3, BMP4, BMP5, GDF-1, and OP-2 in developing and adult tissue are disclosed in co-pending USSN 752,764, and in Ozkaynak, et al., (1991) Biochem. Biophys. Res. Commn. 179:116-123, and Ozkaynak, et al. (1992) (JBC, in press), the disclosures of which are incorporated herein by reference. Using the general probing methodology described herein, northern blot hybridizations using probes specific for these morphogens to probe brain, spleen, lung, heart, liver and kidney tissue indicate that kidney-related tissue appears to be the primary expression source for OP-1, with brain, heart and lung tissues being secondary sources. OP-1 mRNA also was identified in salivary glands, specifically rat parotid glands, using this probing methodology. Lung tissue appears to be the

2.1 Morphogen Detection in Milk

OP-1 was partially purified from rat mammary gland extract and bovine colostrum and 57 day milk by passing these fluids over a series of chromatography columns: (e.g., cation-exchange, affinity and reverse phase). At each step the eluant was collected in fractions and these were tested for the presence of OP-1 by standard immunoblot. Immunoreactive fractions then were combined and purified further. The final, partially purified product then was examined for the presence of OP-1 by Western blot analysis using OP-1-specific antisera, and tested for in vivo and in vitro activity.

OP-1 purified from the different milk sources were characterized by Western blotting using antibodies raised against OP-1 and BMP2. Antibodies were prepared using standard immunology protocols well known in the art, and as described generally in Example 15, below, using full-length E. coli-produced OP-1 and BMP2 as the immunogens. In all cases, the purified OP-1 reacted only with the anti-OP-1 antibody, and not with anti-BMP2 antibody.

The morphogenic activity of OP-1 purified from mammary gland extract was evaluated in vivo essentially following the rat model assay described in U.S. Pat. No. 4,968,590, hereby incorporated by reference. Briefly, a sample was prepared from each OP-1 immunoreactive fraction of the mammary gland extract-derived OP-1 final product by lyophilizing a portion (33%) of the fraction and resuspending the protein in 220$\mu$l of 50% acetonitrile/0.1% TFA. After vortexing, 25 mg of collagen matrix was added. The samples were lyophilized overnight, and implanted in

Long Evans rats (Charles River Laboratories, Wilmington, MA, 28-35 days old). Each fraction was implanted in duplicate. For details of the collagen matrix implantation procedure, see, for example, U.S. Pat. No. 4,968,590, hereby incorporated by reference. After 12 days, the implants were removed and evaluated for new bone formation by histological observation as described in U.S. Patent No. 4,968,590. In all cases, the immunoreactive fractions were osteogenically active.

2.2 Morphogen Detection in Serum

Morphogen may be detected in serum using morphogen-specific antibodies. The assay may be performed using any standard immunoassay, such as Western blot (immunoblot) and the like. Preferably, the assay is performed using an affinity column to which the morphogen-specific antibody is bound and through which the sample serum then is poured, to selectively extract the morphogen of interest. The morphogen then is eluted. A suitable elution buffer may be determined empirically by determining appropriate binding and elution conditions first with a control (e.g., purified, recombinantly-produced morphogen.) Fractions then are tested for the presence of the morphogen by standard immunoblot, and the results confirmed by N-terminal sequencing. Preferably, the affinity column is prepared using monoclonal antibodies. Morphogen concentrations in serum or other fluid samples then may be determined using standard protein quantification techniques, including by spectrophotometric absorbance or by quantitation of conjugated antibody.

Presented below is a sample protocol for identifying OP-1 in serum. Following this general methodology other morphogens may be detected in body fluids, including serum. The identification of morphogen in serum further indicates that systemic administration is a suitable means for providing therapeutic concentrations of a morphogen to an individual, and that morphogens likely behave systemically as endocrine-like factors. Finally, using this protocol, fluctuations in endogenous morphogen levels can be detected, and these altered levels may be used as an indicator of tissue dysfunction. Alternatively, fluctuations in morphogen levels may be assessed by monitoring morphogen transcription levels, either by standard northern blot analysis as described in Example 1, or by in situ hybridization, using a labelled probe capable of hybridizing specifically to morphogen mRNA, and standard RNA hybridization protocols well described in the art and described generally in Example 1.

OP-1 was detected in human serum using the following assay. A monoclonal antibody raised against mammalian, recombinantly produced OP-1 using standard immunology techniques well described in the art and described generally in Example 15, was immobilized by passing the antibody over an agarose-activated gel (e.g., Affi-Gel™, from Bio-Rad Laboratories, Richmond, CA, prepared following manufacturer's instructions) and used to purify OP-1 from serum. Human serum then was passed over the column and eluted with 3M K-thiocyanate. K-thiocyanante fractions then were dialyzed in 6M urea, 20mM $PO_4$, pH 7.0, applied to a C8 HPLC column, and eluted with a 20 minute, 25-50% acetonitrile/0.1% TFA gradient. Mature, recombinantly

produced OP-1 homodimers elute between 20-22 minutes. Fractions then were collected and tested for the presence of OP-1 by standard immunoblot using an OP-1 specific antibody as for Example 2.A.

Administered or endogenous morphogen levels may be monitored in the therapies described herein by comparing the quantity of morphogen present in a body fluid sample with a predetermined reference value, for example, to evaluate the efficiency of a therapeutic protocol, and the like. In addition, fluctuations in the level of endogenous morphogen antibodies may be detected by this method, most likely in serum, using an antibody or other binding protein capable of interacting specifically with the endogenous morphogen antibody. Detected fluctuations in the levels of the morphogen or endogenous antibody may be used, for example, as indicators of a change in tissue status. For example, as damaged tissue is regenerated and the tissue or organ's function returns to "normal" and, in the absence of additional tissue damage, lower doses of morphogen may be required, and a higher level of circulating morphogen antibody may be measured.

Example 3. <u>Effect of Morphogen after the Onset of the Ischemic Process</u>

The cardioprotective effect of morphogens following ischemic-reperfusion injury in a mammal can readily be assessed in a rat model. In this example, morphogen (e.g., OP-1) is administered just prior to the onset of the ischemic process in experimentally-induced myocardial infracted rats, essentially following the method of Lefer, et al. (1990) <u>Science</u> <u>249</u>:61-64 and (1992) <u>J. Mol. Cell. Cardiol.</u> <u>24</u>: 385-393, the

disclosures of which are hereby incorporated by reference. Briefly, loss of myocardial tissue function following ischemia and reperfusion is assayed by measuring loss of myocardial creatine kinease activity (CK) and loss of endothelium-dependent vasorelaxation function (see Example 4, below).

In a first group of ether-anesthetized rats, the left coronary artery was occluded just proximal to the first main branch with a silk ligature to induce a myocardial infarction (MI). The ligature was removed 10 minutes after occlusion to allow for coronary reperfusion. This first group is referred to herein as the "myocardial infarcted" (MI) group. A second group of rats underwent the same procedure except that the coronary artery was not occluded, and thus no myocardial infarction occurred. The second group of rats is referred to herein as the "sham myocardial infarcted group" (SHAM MI).

The first group of rats, the MI group of rats, further was divided into three sup-groups. 2$\mu$g of morphogen (OP-1) were injected intravenously into the first sub-group of MI rats 10 minutes after ligature, immediately before reperfusion; into the second sub-group of MI rats 20 $\mu$g of OP-1 were injected intravenously 10 minutes after ligature and immediately before reperfusion; and into the third sub-group of MI rats (control) was injected vehicle only, e.g., 0.9% NaCl, as for the OP-1 treated rats.

Twenty-four hours later, the hearts were removed from all of the rats and the levels of creatine kinase (CK) from the left ventricle (the infarcted region) and from the interventricular septum (the control

nonischemic region) were determined by standard means. By comparing the difference in CK activities in both regions, the amount of CK activity lost from the infarcted region was used as an index of cardiac cellular injury to the infarcted region.

As shown in Figure 1, the data indicate that morphogens (e.g., OP-1) can provide significant cardioprotective effect when provided to ischemic tissue. In the figure, CK loss is graphed as the difference in specific CK activity between the interventricular septum and the left ventricle.

The loss of CK activity by the subgroup of MI rats which received 2 μg of OP-1 just before reperfusion showed some protection as compared with the control MI rats which received injections of vehicle alone, when the levels from both subgroups are measured against, and compared to, the levels obtained for the SHAM MI control. Significant cardioprotection was observed in the subgroup of MI rats which received 20 μg of OP-1 immediately before reperfusion as compared with the control MI rats which received injections of vehicle alone, when the levels from both subgroups are measured against, and compared to, the levels contained within the SHAM MI control.

These data indicate that OP-1 offers significant cardiac protection when administered after ischemia and before reperfusion.

A variation of this example also may be performed providing morphogen to the animal prior to induction of ischemia. The experiments may be performed both in normal and immune-compromised rats to assess the cardioprotective effects of morphogen administered prior to ischemia.

Example 4. Vasodilation of Myocardial Infarcted Cardiac Tissue Treated with Morphogen

Certain vasodilators like acetylcholine (ACh) and adenosine diphosphate (ADP, an immune mediator) exert their vasodilation activity only in the presence of intact endothelium, which is stimulated to release a substance termed endothelium-derived relaxing factor (EDRF). If the endothelium is injured so that EDRF is not released, no vasodilation occurs in response to these endothelium-dependent agents. In contrast, several other vasodilators including nitroglycerine (NTG) and nitroprusside, are endothelium-independent dilators, as they dilate blood vessels directly.

The present example demonstrates the ability of OP-1 to prevent the loss of cardioendothelium-dependent relaxation (EDR) activity in the coronary microvasculature following reperfusion of ischemic myocardium, and their ability to reduce myocardial injury 24 hours after morphogen treatment. Briefly, 2 or 24 hours after morphogen treatment ischemia-reperfusion injury is induced in isolated rat hearts, the reperfused hearts are are vasodilated with either ACh or NTG. In the absence of morphogen treatment, injured tissue should inhibit ACh-induced vasodilation, but not NTG-induced vasodilation. Morphogen treatment in expected to enhance ACh-induced vasodilation in the reperfused hearts.

Accordingly, 48 adult male Sprague-Dawley rats (250-330 g) were divided into eight groups of 6 rats each. Twelve rats were subjected to sham myocardial infarcts (SHAM MI) as described in Example 3. The hearts of the remaining 36 rats were isolated as follows: one set of twelve rats was injected intravenously with OP-1 24 hours prior to isolation of the heart; another set of rats was injected intravenously with 20$\mu$g of OP-1 2 hours prior to isolation of the heart; the final group of rats was injected with vehicle only (e.g., 0.9% NaCl.). The rats then were anesthetized with pentobarbital sodium (35 mg/kg, intraperitonial); their hearts were isolated and perfused by the Langendorff method at a constant flow (15 ml/min) with oxygenated Krebs-Henseleit solution (Aoki et al. (1988) J. Pharmacol. 95:35).

Each group of rats then were divided into two subgroups of six rats each. Twenty minutes before reperfusion, coronary vasodilator response was measured by inducing constriction with 0.05 $\mu$mol U-44619 (9,11-methanoepoxyprostaglandin $H_2$) followed by a vasodilating agent 3 minutes later: subgroup one - 15 nmol ACh; subgroup 2 - 15 nmol NTG and the increase in coronary perfusion pressure (CPP) level measured as an indication of vasodilation. When CPP levels returned to normal, the hearts were subjected to ischemia by reducing coronary infusion to 15% of control flow for 30 minutes, then reestablishing normal flow, i.e., reperfusion, for an additional 20 minutes.

The vasodilator reponse then was remeasured by constriction and administration of vasodilating agent as described above.

The results of these experiments are shown in FIG 2. Before the ischemic event, both Ach and NTG gave normal vasorelaxant results in all events. The hearts which received OP-1 24 hours prior to ischemia showed an approximately 70% response to ACh while the hearts which received OP-1 2 hours prior to ischemia showed a 55% response to ACh. The group which received vehicle alone showed a 40% response to ACh. Finally, the control group which was not subjected to ischemia showed an ACh response of approximately 95%. This shows that endothelium-dependent vasodilators exert a reduced vasodilator response following ischemia and reperfusion in the rat heart. Moreover, OP-1 significantly preserved endothelium-dependent dilation when provided 24 hours prior to induction of myocardial ischemia. No defect in vasodilation occurred in response to the direct vasodilator (NTG); NTG-induced vasodilation activities were 95% of initial in hearts subject to ischemia and 100% of initial nonischemic hearts.

Example 5. Effect of Morphogen on Neutrophil Adherence

The role of neutrophil adherence in endothelium dysfunction and the cardioprotective effects of morphogens in modulating this activity can be assessed using a standard polymorphonuclear neutrophil (PMN) adherence assay such as described in Lefer et al., (1992) J. Mol. Cell. Cardiol. 24: 385-393, disclosed hereinabove by reference. Briefly, segments of superior mesenteric artery were isolated from rats which had either been treated with morphogen (OP-1, 20 μg) or 0.9% NaCl, 24 h prior to isolation of the artery. The segments were cleaned, cut into transverse rings of 1-2mm in length, and these were subsequently

cut open and incubated in K-H solution at 37°C, pH 7.4. Neutrophils were prepared and fluorescently labelled using standard procedures (e.g., leukocytes were isolated from rats essentially following the procedure of Pertroft et. al. (1968) Exp Cell Res 50: 355-368, washed in phosphate buffered saline (PBS), purified by gradient centrifugation; and labelled by the method of Yuan et. al. (1990) Microvasc Res 40: 218-229..

Labelled neutrophils then were added to open ring baths and activated with 100nM leukotriene $B_4$ ($LTB_4$). Rings were incubated for 20 minutes and the number of neutrophils adhering to the endothelial surface then determined visually by fluorescent microscopy.

As shown in Figure 3, unstimulated PMNs (i.e., PMNs alone) added to the baths did not significantly adhere to the vascular endothelium. In rings taken from rats injected with 0.9% NaCl, activation of neutrophils with $LTB_4$ (100 nM) greatly increased the number of PMNs adherent to the endothelium ($P<0.001$). OP-1 (20 μg administered 24 h prior) significantly inhibited adherence of PMNs activated by $LTB_4$ ($P<0.01$ from control).

Example 6. In Vivo Models for Ischemic-Reperfusion Protection in Lung, Nerve and Renal Tissue.

Other tissues seriously affected by ischemic-reperfusion injury include neural tissue, renal tissue and lung tissue. The effect of morphogens on alleviating the ischemic-reperfusion injury in these tissues may be assessed using methodologies and models known to those skilled in the art, and disclosed below.

Similarly, a methodology also is provided for assessing the tissue-protective effects of a morphogen on damaged lung tissue following hyperoxia injury.

For example, the rabbit embolic stroke model provides a useful method for assessing the effect of morphogens on tissue injury following cerebral ischemia-reperfusion. The protocol disclosed below is essentially that of Phillips et al. (1989) Annals of Neurology 25:281-285, the disclosure of which is herein incorporated by reference. Briefly, white New England rabbits (2-3kg) are anesthesized and placed on a respirator. The intracranial circulation then is selectively catheterized by the Seldinger technique. Baseline cerebral angiography then is performed, employing a digital substration unit. The distal internal carotid artery or its branches then is selectively embolized with 0.035 ml of 18-hour-aged autologous thrombus. Arterial occlusion is documented by repeat angiography immediately after embolization. After a time sufficient to induce cerebral infarcts (15 minutes or 90 minutes), reperfusion is induced by administering a bolus of a reperfusion agent such as the TPA analogue Fb-FB-CF (e.g., 0.8 mg/kg over 2 minutes).

The effect of morphogen on cerebral infarcts can be assessed by administering varying concentrations of morphogens, e.g., OP1, at different times preceding or following embolization and/or reperfusion. The rabbits are sacrificed 3-14 days post embolization and their brains prepared for neuropathological examination by fixing by immersion in 10% neutral buffered formalin

for at least 2 weeks. The brains then are sectioned in a coronal plane at 2-3 mm intervals, numbered and submitted for standard histological processing in paraffin, and the degree of neutral tissue necrosis determined visually.

The renal-protective effects of morphogens on renal ischemia-reperfusion injury readily can be assessed using the mouse model disclosed by Oueliette, et al. (1990), J. Clin. Invest. 85:766-771, the disclosure of which is hereby incorporated by reference. Briefly, renal ischemia is induced surgically in 35-45 days old out-bred Swiss male mice by performing a standard right nephrectomy, and occluding the artery to the left kidney with a microaneurism clamp for 10-30 minutes. Morphogen then may be provided parentally, at various times prior to or following occulsion and/or reperfusion. The effects of morphogen then may be assessed by biological evaluation and histological evaluation using standard techniques well known in the art.

The tissue protective effects of morphogen on tissue exposed to lethally high oxygen concentrations may be assessed by the following procedure. Adult rats (275-300 gms) first are provided with morphogen (e.g., hOP1) or vehicle only, and then are exposed to 96-98% oxygen essentially as described by Rinaldo et al (1983) Am. Rev. Respir. Dis. 130:1065, to induce hyperoxia. Animals are housed in plastic cages (38 cm x 48 xm x 21 cm). A cage containing 4-5 animals is placed in a 75 liter water-sealed plexiglass chamber. An atmosphere of 96-98% oxygen then is maintained by delivery of $O_2$ gas (liquid $O_2$). Gas flow through the chamber is adjusted to maintain at least 10 air changes/hr.,

temperature at $22 \pm 1^{\circ}C$, minimal levels of condensation within the cage, and carbon dioxide concentration of < 0.5% as measured with a mass spetrophotometric medical gas analyzer.

At the end of 72 hours all survivors are observed at room air for 1.5 hours and at longer time periods to assess degree of respiratory distress and cyanosis induced by the initial insult and subsequent immune cell-mediated damage. The number of survivors at the end of the challenge is recorded and the treated groups compared with the untreated control group by chi-square test of proportions. Several of the surviving animals for each group are randomly chosen for histological processing of lung tissue.

Lung tissue for histological processing is fixed by infusion of 10% buffered formalin through a tracheal cannula at a constant pressure of 20 cm $H_2O$. After fixation for 24-48 hours, sections from each lobe are cut and subsequently stained with hematoxylin and eosin. Coded slides then are examined, preferably in a double-blind fashion for evidence of pathological changes such as edema, interstitial cellularity, and inflammatory response.

Example 7. <u>Morphogen Inhibition of Cellular and Humoral Inflammatory Response</u>

Morphogens described herein inhibit multinucleation of mononuclear phagocytic cells under conditions where these cells normally would be activated, e.g., in response to a tissue injury or the presence of a foreign substance. For example, in the absence of morphogen, an implanted substrate material (e.g.,

implanted subcutaneously) composed of, for example, mineralized bone, a ceramic such as titanium oxide or any other substrate that provokes multinucleated giant cell formation, rapidly becomes surrounded by multinucleated giant cells, e.g., activated phagocytes stimulated to respond and destroy the foreign object. In the presence of morphogen however, the recruited cells remain in their mononuclear precursor form and the matrix material is undisturbed. Figure 4 illustrates this effect of morphogens, in a schematic representation of histology results of a titanium oxide substrate implanted subcutaneously. In the figure, "mg" means mononuclear giant cells and "ob" means osteoblasts. The substrate represented in Fig. 4B was implanted together with morphogen (OP-1) and newly formed osteoblasts are evident surrounding the substrate. By contrast, the substrate represented in Fig. 4A was implanted without morphogen and extensive multinucleated giant cell formation is evident surrounding the substrate. Accordingly, the morphogens' effect in inhibiting excessive bone mass loss in a mammal also may include inhibiting activation of these cells.

In addition, the morphogens described herein also suppress antibody production stimulated in response to a foreign antigen in a mammal. Specifically, when bovine bone collagen matrix alone was implanted in a bony site in a rat, a standard antibody response to the collagen is stimulated in the rat as determined by standard anti-bovine collagen ELISA experiments performed on blood samples taken at four week intervals following implantation (e.g., between 12 and 20 weeks.) Serum anti-collagen antibody titers, measured by ELISA essentially following the procedure described by

Nagler-Anderson et al, (1986) PNAS 83:7443-7446, the disclosure of which is incorporated herein by reference, increased consistently throughout the experiment. However, when the matrix was implanted together with a morphogen (e.g., OP-1, dispersed in the matrix and adsorbed thereto, essentially as described in U.S. Pat. No. 4,968,590) anti-bovine collagen antibody production was suppressed significantly. This ability of morphogen to suppress the humoral response is further evidence of morphogen utility in alleviating tissue damage associated with autoimmune diseases, including autoantibody diseases, such as rheumatoid arthritis.

Example 8. Morphogen protection of Gastrointestinal Tract Mucosa from Ulceration and Inflammation

Oral mucositis is a gastrointestinal tract inflammatory disease which involves ulcerations of the mouth mucosa as a consequence of, e.g., radiation therapy or chemotherapy. While not typically a chronic disease, the tissue destructive effects of oral mucositis mirror those of chronic inflammatory diseases such as IBD. The example below demonstrates morphogen efficacy in protecting the oral mucosa from oral mucositis in a hamster model, including both inhibiting inflammatory ulceration and enhancing regeneration of ulcerated tissue. Details of the protocol can be found in Sonis, et al., (1990) Oral Surg. Oral Med. Oral Pathol 69: 437-443, the disclosure of which is incorporated herein by reference. Based on these data,

the morphogens described herein should be efficacious in treating chronic inflammatory diseases including IBD, arthritis, psoriasis and psoriatic arthritis, multiple sclerosis, and the like.

Golden syrian hamsters (6-8 wks old, Charles River Laboratories, Wilmington, MA) were divided into 3 test groups: Group 1, a placebo (e.g., saline) control, and a morphogen low dose group (100 ng) and a morphogen high dose group (1 μg), Groups 2 and 3, respectively. Morphogen dosages were provided in 30% ethanol. Each group contained 12 animals.

Beginning on day 0 and continuing through day 5, Groups 2 and 3 received twice daily morphogen applications. On day 3, all groups began the mucositis-induction procedure. 5-fluorouracil (60 mg/kg) was injected intraperitoneally on days 3 and 5. On day 7, the right buccal pouch mucosa was superficially irritated with a calibrated 18 gauge needle. In untreated animals, severe ulcerative mucositis was induced in at least 80% of the animals by day 10.

For each administration of the vehicle control (placebo) or morphogen, administration was performed by first gently drying the cheek pouch mucosa, then providing an even application over the mucosal surface of the vehicle or morphogen material. A hydroxypropylcellulose-based coating was used to maintain contact of the morphogen with the mucosa. This coating provided at least 4 hours of contact time.

On day 12, two animals in each group were sacrificed for histological studies. The right buccal pouch mucosa and underlying connective tissue were dissected and fixed in 10% formalin using standard dissection and histology procedures. The specimens were mounted in paraffin and prepared for histologic examination. Sections then were stained with hematoxylin and eosin and were examined blindly by three oral pathologists with expertise in hamster histology and scored blind against a standard mucositis panel. The extent of atrophy, cellular infiltration, connective tissue breakdown, degree of ulceration and epithelialization were assessed.

The mean mucositis score for each group was determined daily for each experimental group for a period of 21 days by photography and visual examination of the right buccal cheek pouch. Differences between groups were determined using a standard 't' test, e.g., the Students' 't' test. In addition, data was evaluated between groups by comparing the numbers of animals with severe mucositis using Chi Square statistical analysis. The significance of differences in mean daily weights also was determined.

The experimental results are presented in Fig. 5, which graphs the effect of morphogen (high dose, squares; low dose, diamonds) and placebo (circles) on mean mucositis scores. Both low and high morphogen doses inhibit lesion formation significantly in a dose-dependent manner. In addition, histology results consistently showed significantly reduced amounts of

tissue atrophy, cellular debris, and immune effector cells, including macrophages and activated neutrophils, in the morphogen-treated animals, as compared with the untreated, control animals.

Example 9. Morphogen Effect on Fibrogenesis and Scar Tissue Formation

The morphogens described herein induce tissue morphogenesis of damaged or lost tissue. The ability of these proteins to regenerate new tissue enhances the anti-inflammatory effect of these proteins. Provided below are a series of in vitro experiments demonstrating the ability of morphogens to induce migration and accumulation of mesenchymal cells. In addition, the experiments demonstrate that morphogens, unlike TGF-β, do not stimulate fibrogenesis or scar tissue formation. Specifically, morphogens do not stimulate production of collagen, hyaluronic acid (HA) or metalloproteinases in primary fibroblasts, all of which are required for fibrogenesis or scar tissue formation. By contrast, TGF-β, a known inducer of fibrosis, but not of tissue morphogenesis, does stimulate production of these fibrosis markers.

Chemotaxis and migration of mesenchymal progenitor cells were measured in modified Boyden chambers essentially as described by Fava, R.A. et al (1991) J. Exp. Med. 173: 1121-1132, the disclosure of which is incorporated herein by reference, using polycarbonate filters of 2, 3 and 8 micron ports to measure migration of progenitor neutrophils, monocytes and fibroblasts. Chemotaxis was measured over a range of morphogen concentrations, e.g., $10^{-20}$M to $10^{-12}$M OP-1. For progenitor neutrophils and monocytes, $10^{-18}$-$10^{-17}$M OP-1

consistently induced maximal migration, and $10^{-14}$ to $10^{-13}$M OP-1 maximally induced migration of progenitor fibroblasts. In all cases the chemotactic activity could be inhibited with anti-OP-1 antibody. Similar migration activities also were measured and observed with TGF-β.

The effect of morphogen on fibrogenesis was determined by evaluating fibroblast production of hyaluronic acid (HA), collagen, collagenese and tissue inhibitor of metalloproteinases (TIMP).

Human fibroblasts were established from explants of infant foreskins and maintained in monolayer culture using standard culturing procedures. (See, for example, (1976) J. Exp. Med. 144: 1188-1203.) Briefly, fibroblasts were grown in maintenance medium consisting of Eagle's MEM, supplemented with nonessential amino acids, ascorbic acid (50 μg/ml), $NaHCO_3$ and HEPES buffers (pH 7.2), penicillin (100 U/ml), streptomycin (100 μg/ml), amphotericin B (1 μg/ml) and 9% heat inactivated FCS. Fibroblasts used as target cells to measure chemotaxis were maintained in 150 mm diameter glass petri dishes. Fibroblasts used in assays to measure synthesis of collagen, hyaluronic acid, collagenase and tissue inhibitors of metalloproteinases (TIMP) were grown in 100 mm diameter plastic tissue culture petri dishes.

The effects of morphogen on fibroblast production of hyaluronic acid, collagens, collagenase and TIMP were determined by standard assays (See, for example, Posttethwaite et al. (1989) J. Clin. Invest. 83: 629-636, Posttethwaithe (1988) J./ Cell Biol. 106: 311-318 and Clark et al (1985) Arch. Bio-chem Biophys. 241: 36-

44, the disclosures of which are incorporated by reference.) For these assays, fibroblasts were transferred to 24-well tissue culture plates at a density of 8 x $10^4$ cells per well. Fibroblasts were grown confluency in maintenance medium containing 9% FCS for 72 h and then grown in serum-free maintenance medium for 24 h. Medium was then removed from each well and various concentrations of OP-1 (recombinantly produced mature or soluble form) or TGF-β-1 (R&D Systems, Minneapolis) in 50 μl PBS were added to triplicate wells containing the confluent fibroblast monolayers. For experiments that measured production of collagenase and TIMP, maintenance medium (450 μl) containing 5% FCS was added to each well, and culture supernatants were harvested from each well 48 h later and stored at -70°C until assayed. For experiments that assessed HA production, maintenance medium (450 μl) containing 2.5% FCS was added to each well, and cultures grown for 48 h. For experiments that measured fibroblast production of collagens, serum-free maintenance medium (450 μl) without non-essential amino acids was added to each well and cultures grown for 72 h. Fibroblast production of HA was measured by labeling newly synthesized glycosaminoglycans (GAG) with [$^3$H]-acetate the last 24 h of culture and quantitating released radioactivity after incubation with hyaluronidase from Streptomyces hyalurolyticus (ICN Biochemicals, Cleveland, OH) which specifically degrades hyaluronic acid. Production of total collagen by fibroblasts was measured using a collagenase-sensitive protein assay that reflects [$^3$H]-proline incorporation the last 24 h of culture into newly synthesized collagens. Collagenase and TIMP protein levels in fibroblast cultures supernatants was measured by specific ELISAs.

As shown in Fig. 6, OP1 does not stimulate significant collagen or HA production, as compared with TGF-β. In the figure, panel A shows OP-1 efect on collagen production, panel B shows TGF-β effect on collagen production, and panels C and D show OP-1 (panel C) and TGF-β (panel D) effect on HA production. The morphogen results were the same whether the soluble or mature form of OP1 was used. By contrast, the latent form of TGF-β (e.g., pro domain-associated form of TGF-β) was not active.

Example 10. Morphogen Inhibition of Epithelial Cell Proliferation

This example demonstrates the ability of morphogens to inhibit epithelial cell proliferation *in vitro*, as determined by $^{3}H$-thymidine uptake using culture cells from a mink lung epithelial cell line (ATCC No. CCL 64), and standard mammalian cell culturing procedures. Briefly, cells were grown to confluency in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS), 200 units/ml penicillin, and 200 μg/ml streptomycin, and used to seed a 48-well cell culture plate at a cell density of 200,000 cells per well. When this culture became confluent, the media was replaced with 0.5 ml of EMEM containing 1% FBS and penicillin/streptomycin and the culture incubated for 24 hours at 37 C. Morphogen test samples in EMEM containing 5% FBS then were added to the wells, and the cells incubated for another 18 hours. After incubation, 1.0 μCi of $^{3}H$-thymidine in 10 μl was added to each well, and the cells incubated for four hours at 37 C. The media then was removed and the cells washed once with ice-cold phosphate-buffer saline and DNA precipitated by adding 0.5 ml of 10% TCA to each well

and incubating at room temperature of 15 minutes. The cells then were washed three times with ice-cold distilled water, lysed with 0.5 ml 0.4 M NaOH, and the lysate from each well then transferred to a scintillation vial and the radioactivity recorded using a scintillation counter (Smith-Kline Beckman).

The results are presented in Table III, below. The anti-proliferative effect of the various morphogens tested was expressed as the counts of 3H-thymidine (x 1000) integrated into DNA, and were compared with untreated cells (negative control) and TGF-β (1 ng), a local-acting factor also known to inhibit epithelial cell proliferation. COP-5 and COP-7 are biosynthetic constructs that previously have been shown to have osteogenic activity, capable of inducing the complete cascade resulting in endochondral bone formation in a standard rat bone assay (see U.S. Pat. No. 5,011,691.) The morphogens significantly inhibit epithelial cell proliferation. Similar experiments, performed with the morphogens COP-16, bOP (bone-purified osteogenic protein, a dimeric protein comprising CBMP2 and OP-1), and recombinant OP-1, also inhibit cell proliferation. bOP and COP-16 also induce endochondral bone formation (see US Pat. No. 4,968,590 and 5,011,691.)

TABLE III

| | Thymidine uptake (x 1000) |
|---|---|
| control | 50.048, 53.692 |
| COP-7-1 (10 ng) | 11.874 |
| COP-7-2 (3 ng) | 11.136 |
| COP-5-1 (66 ng) | 16.094 |
| COP-5-2 (164 ng) | 14.43 |
| TGF-β (1 ng) | 1.86, 1.478 |

Example 11. Morphogen Treatment of a Systemic Inflammatory Disease

The following example provides a rat adjuvant-induced arthritis model for demonstrating morphogen efficacy in treating arthritis and other systemic inflammatory diseases. Rat adjuvant-induced arthritis induces a systemic inflammatory disease with bone and cartilage changes similar to those observed in rhematoid arthritis, but in an accelerated time span (see, for example, Pearson (1964) Arth. Rheum. 7:80). A detailed description of the protocol is provided in Walz, et al., (1971) J. Pharmac. Exp. Ther. 178: 223-231, the disclosure of which is incorporated herein by reference.

Briefly, Sprague-Dawley female rats (e.g., Charles River Laboratories, Wilmington, MA) are randomized into 3 groups: control; morphogen, low dose (e.g., 1-10 μg/kg weight per day) and morphogen, high dose (e.g., 10-20 μg/kg weight per day), referred to as Groups 1, 2, and 3, respectively.

Adjuvant arthritis is induced in all three groups by injection of 0.05 ml of a suspension of 1.5% dead Mycobacterium butyricum in mineral oil into the subplantar surface of the right hand paw. On Day 18 after adjuvant injection, the limb volumes of both hind limb are determined. In the absence of morphogen treatment, a systemic arthritic condition is induced in adjuvant-injected rats by this time, as determined by significant swelling of the uninjected hind limbs (< 2.3 ml, volume measured by mercury displacement).

Subsequent determinations of paw edema and x-ray scores are made on the uninjected hind limb. Rats in Group 2 and 3 also are dosed orally daily, beginning on Day 1, with morphogen. Limb volumes are recorded on Days 29 and 50 after adjuvant injection and edema determined by volume difference compared to Day 18. The uninjected hind limb on each rat is x-rayed on Day 50 and the joint damage assayed on an arbitrary scale of 1 to 10 (1=no damage, 10=maximum damage). Data on differences between control and treated groups (Day 29 edema, Day 50 edema and Day 50 x-ray scores) are analyzed by using a standard "t-test. Morphogen-treated rats show consistently reduced joint damage (e.g., decreased in edema and in x-ray scores) as compared with untreated control rats.

As another, alternative example, Groups 2 and 3 are dosed daily with morphogen beginning on Day 18 and continuing through Day 50 to demonstrate the efficacy of morphogens in arthritic animals.

Example 12. <u>Morphogen Inhibition of Localized Edema</u>

The following example demonstrates morphogen efficacy in inhibiting a localized inflammatory response in a standard rat edema model. Experimental rats (e.g., Long-Evans from Charles River Laboratories, Wilmington, MA) are divided into three groups: Group 1, a negative control, which receives vehicle alone; Group 2, a positive control, to which is administered a well-known characterized anti-inflammatory agent (e.g., indomethacin), and Group 3, to which morphogen is provided.

Groups 2 and 3 may be further subdivided to test low, medium and high doses (e.g., Group 2: 1.0 mg/kg, 3.0 mg/kg and 9.0 mg/kg indomethacin; Group 3: 0.1-5μg; 5-20μg, and 20-50μg of morphogen). Sixty minutes after indomethacin or morphogen is provided to the rats of Group 2 or 3 (e.g., as by injection into the tail vein, or by oral gavage) inflammation is induced in all rats by a sub-plantar injection of a 1% carrageenin solution (50μl) into the right hind paw. Three hours after carrageenin administration paw thickness is measured as an indication of edema (e.g., swelling) and induced inflammatory response to the injected carrageenin solution.

Significant swelling is evident in untreated rats by three hours after carrageenin injection. Inflammation also is measured by histology by standard means, following euthanasia e.g.: the right hind paw from each animal is removed at the ankle joint and weighed and foot pad tissue is fixed in 10% neutral buffered formalin, and slides prepared for visual examination by staining the prepared tissue with hematoxylin and eosin.

The morphogen-treated rats show substantially reduced edema induction following carrageenin injection as compared with the untreated rats.

Example 13. Morphogen Treatment of Allergic Encephalomyelitis

The following example demonstrates morphogen efficacy in treating experimental allergic encephalomyelitis (EAE) in a rat. EAE is a well-characterized animal model for multiple sclerosis, an autoimmune disease. A detailed description of the protocol is disclosed in Kuruvilla, et al., (1991) PNAS 88:2918-2921, the disclosure of which is incorporated herein by reference.

Briefly, EAE is induced in rats (e.g., Long-Evans, Charles River Laboratories, Wilmington, MA) by injection of a CNS tissue (e.g., spinal cord) homogenate in complete Freund's adjuvant (CFA) on days -44, -30 and 0 (last day of immunization), by subcutaneous injection to three sites on the animal's back. Morphogen is administered daily by interperitoneal injection beginning on day -31. Preferably, a series of morphogen dose ranges is evaluated (e.g., low, medium and high) as for Example 12, above.) Control rats receive morphogen vehicle only (e.g. 0.9% NaCl or buffered saline). Rats are examined daily for signs of disease and graded on an increasing severity scale of 0-4.

In the absence of morphogen treatment, significant neurological dysfunction (e.g., hind and fore limb weakness, progressing to total hind limb paralysis) is evident by day +7 to +10. Hematology, serum chemistry profiles and histology are performed to evaluate the

degree of tissue necropsy using standard procedures. Morphogen treatment significantly inhibits the neurological dysfunction normally evident in an EAE animal. In addition, the histopathological markers typically associated with EAE are absent in the morphogen-treated animals.

Example 14. Morphogen Treatment of Collagen-Induced Arthritis

The following example demonstrates the efficacy of morphogens in inhibiting the inflammatory response in a collagen-induced arthritis (CIA) in a rat. CIA is a well-characterized animal model for rheumatoid arthritis, an autoimmune disease. The protocol disclosed is essentially that disclosed in Kuruvilla et al., (1991) PNAS 88:2918-2921, incorporated by reference hereinabove. Briefly, CIA is induced in experimental rats (e.g., Long-Evans, Charles River Laboratories, Wilmington), by multiple intradermal injection of bovine Type II collagen (e.g., 100μg) in CFA (0.2 ml) on Day 1. Animals are divided into two groups: Group 1, control animals, which receive vehicle alone, and Group 2: morphogen-treated animals, which, preferably, are subdivided into low, medium and high dose ranges, as described for Example 13, above. Morphogen is administered daily (e.g., by tail vein injection) beginning at different times following collagen injection, e.g., beginning on day 7, 14, 28, 35 and 42. Animals are evaluated visually and paw thickness and body weight is monitored throughout the experiment. Animals are sacrificed on day 60 and the proximal and distal limb joints, and ear, tail and spinal cord prepared for histological evaluation as described for Examples 12 and 13, above. In a

variation of the experiment, morphogen may be administered for prescribed periods, e.g., five day periods, beginning at different times following collagen injection (e.g., on days 0-4, 7-11, 14-18, 28-32.)

In the absence of morphogen treatment, an arthritic condition typically is induced by 30 days post collagen injection. In morphogen-treated animals, CIA is suppressed and the histopathological changes typically evidenced in control CIA-induced animals are absent: e.g., accumulations of activated mononuclear inflammatory cells and fibrous connective tissue. In addition, consistent with the results in Example 7, above, serum anti-collagen antibody titers are suppressed significantly in the morphogen-treated animals.

Example 15. Screening Assay for Candidate Compounds which Alter Endogenous Morphogen Levels

Candidate compound(s) which may be administered to affect the level of a given morphogen may be found using the following screening assay, in which the level of morphogen production by a cell type which produces measurable levels of the morphogen is determined with and without incubating the cell in culture with the compound, in order to assess the effects of the compound on the cell. This can be accomplished by detection of the morphogen either at the protein or RNA level. A more detailed description also may be found in USSN 752,861, incorporated hereinabove by reference.

## 15.1 Growth of Cells in Culture

Cell cultures of kidney, adrenals, urinary bladder, brain, or other organs, may be prepared as described widely in the literature. For example, kidneys may be explanted from neonatal or new born or young or adult rodents (mouse or rat) and used in organ culture as whole or sliced (1-4 mm) tissues. Primary tissue cultures and established cell lines, also derived from kidney, adrenals, urinary, bladder, brain, mammary, or other tissues may be established in multiwell plates (6 well or 24 well) according to conventional cell culture techniques, and are cultured in the absence or presence of serum for a period of time (1-7 days). Cells may be cultured, for example, in Dulbecco's Modified Eagle medium (Gibco, Long Island, NY) containing serum (e.g., fetal calf serum at 1%-10%, Gibco) or in serum-deprived medium, as desired, or in defined medium (e.g., containing insulin, transferrin, glucose, albumin, or other growth factors).

Samples for testing the level of morphogen production includes culture supernatants or cell lysates, collected periodically and evaluated for OP-1 production by immunoblot analysis (Sambrook et al., eds., 1989, Molecular Cloning, Cold Spring Harbor Press, Cold Spring Harbor, NY), or a portion of the cell culture itself, collected periodically and used to prepare polyA+ RNA for RNA analysis. To monitor *de novo* OP-1 synthesis, some cultures are labeled according to conventional procedures with an $^{35}$S-methionine/$^{35}$S-cysteine mixture for 6-24 hours and then evaluated to OP-1 synthesis by conventional immunoprecipitation methods.

15.2 Determination of Level of Morphogenic Protein

In order to quantitate the production of a morphogenic protein by a cell type, an immunoassay may be performed to detect the morphogen using a polyclonal or monoclonal antibody specific for that protein. For example, OP-1 may be detected using a polyclonal antibody specific for OP-1 in an ELISA, as follows.

1 μg/100 μl of affinity-purified polyclonal rabbit IgG specific for OP-1 is added to each well of a 96-well plate and incubated at 37°C for an hour. The wells are washed four times with 0.167M sodium borate buffer with 0.15 M NaCl (BSB), pH 8.2, containing 0.1% Tween 20. To minimize non-specific binding, the wells are blocked by filling completely with 1% bovine serum albumin (BSA) in BSB and incubating for 1 hour at 37°C. The wells are then washed four times with BSB containing 0.1% Tween 20. A 100 μl aliquot of an appropriate dilution of each of the test samples of cell culture supernatant is added to each well in triplicate and incubated at 37°C for 30 min. After incubation, 100 μl biotinylated rabbit anti-OP-1 serum (stock solution is about 1 mg/ml and diluted 1:400 in BSB containing 1% BSA before use) is added to each well and incubated at 37°C for 30 min. The wells are then washed four times with BSB containing 0.1% Tween 20. 100 μl strepavidin-alkaline (Southern Biotechnology Associates, Inc. Birmingham, Alabama, diluted 1:2000 in BSB containing 0.1% Tween 20 before use) is added to each well and incubated at 37°C for 30 min. The plates are washed four times with 0.5M Tris buffered Saline (TBS), pH 7.2. 50μl substrate (ELISA Amplification System Kit, Life Technologies, Inc., Bethesda, MD) is added to each well incubated at room temperature for 15

min. Then, 50 $\mu$l amplifier (from the same amplification system kit) is added and incubated for another 15 min at room temperature. The reaction is stopped by the addition of 50 $\mu$l 0.3 M sulphuric acid. The OD at 490 nm of the solution in each well is recorded. To quantitate OP-1 in culture media, a OP-1 standard curve is performed in parallel with the test samples.

Polyclonal antibody may be prepared as follows. Each rabbit is given a primary immunization of 100 ug/500 $\mu$l E. coli produced OP-1 monomer (amino acids 328-431 in SEQ ID NO:5) in 0.1% SDS mixed with 500 $\mu$l Complete Freund's Adjuvant. The antigen is injected subcutaneously at multiple sites on the back and flanks of the animal. The rabbit is boosted after a month in the same manner using incomplete Freund's Adjuvant. Test bleeds are taken from the ear vein seven days later. Two additional boosts and test bleeds are performed at monthly intervals until antibody against OP-1 is detected in the serum using an ELISA assay. Then, the rabbit is boosted monthly with 100 $\mu$g of antigen and bled (15 ml per bleed) at days seven and ten after boosting.

Monoclonal antibody specific for a given morphogen may be prepared as follows. A mouse is given two injections of E. coli produced OP-1 monomer. The first injection contains 100$\mu$g of OP-1 in complete Freund's adjuvant and is given subcutaneously. The second injection contains 50 $\mu$g of OP-1 in incomplete adjuvant and is given intraperitoneally. The mouse then receives a total of 230 $\mu$g of OP-1 (amino acids 307-431 in SEQ ID NO:5) in four intraperitoneal injections at various times over an eight month period. One week

prior to fusion, both mice are boosted intraperitoneally with 100 $\mu$g of OP-1 (307-431) and 30 $\mu$g of the N-terminal peptide ($Ser_{293}$-$Asn_{309}$-Cys) conjugated through the added cysteine to bovine serum albumin with SMCC crosslinking agent. This boost was repeated five days (IP), four days (IP), three days (IP) and one day (IV) prior to fusion. The mouse spleen cells are then fused to myeloma (e.g., 653) cells at a ratio of 1:1 using PEG 1500 (Boeringer Mannheim), and the cell fusion is plated and screened for OP-1-specific antibodies using OP-1 (307-431) as antigen. The cell fusion and monoclonal screening then are according to standard procedures well described in standard texts widely available in the art.

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: KUBERASAMPATH, THANGAVEL
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(ii) TITLE OF INVENTION: MORPHOGEN-INDUCED MODULATION OF INFLAMMATORY RESPONSE

(iii) NUMBER OF SEQUENCES: 33

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(E) COUNTRY: U.S.A.
(F) ZIP:

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Patent In Release #1.0, Version #1.25

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 667,274
(B) FILING DATE: 11-MAR-1991

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 753,059
(B) FILING DATE: 30-AUG-1991

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 752,764
(B) FILING DATE: 30-AUG-1991

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME: Generic Sequence 1
(D) OTHER INFORMATION: Each Xaa indicates one of the 20 naturally-occurring L-isomer, α-amino acids or a derivative thereof.
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
         20                  25
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
     30                  35
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
40                   45                  50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
                 55                  60
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
             65                  70
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
         75                  80
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
     85                  90
Xaa Cys Xaa
 95
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(ix) FEATURE:
(A) NAME: Generic Sequence 2
(D) OTHER INFORMATION: Each Xaa indicates one of the 20 naturally-occurring L-isomer, α-amino acids or a derivative thereof.
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
                              Xaa Xaa Xaa Xaa Xaa Xaa
                               1               5
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
             10                  15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
         20                  25
Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
     30                  35
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
40                   45                  50
```

```
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
                 55                      60
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
             65                      70
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
         75                      80
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
     85                      90
Xaa Cys Xaa
 95
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME: Generic Sequence 3
(D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
    Leu Tyr Val Xaa Phe
    1               5
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                 10
Xaa Ala Pro Gly Xaa Xaa Xaa Ala
 15                  20
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
         25                  30
Xaa Pro Xaa Xaa Xaa Xaa Xaa
                 35
Xaa Xaa Xaa Asn His Ala Xaa Xaa
         40                  45
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
                 50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
     55                  60
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
             65
Xaa Xaa Xaa Leu Xaa Xaa Xaa
 70                  75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
             80
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
 85                  90
Xaa Cys Gly Cys Xaa
         95
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(ix) FEATURE:
(A) NAME: Generic Sequence 4
(D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Cys Xaa Xaa Xaa Xaa Leu Tyr Val Xaa Phe
 1              5                   10
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                15
Xaa Ala Pro Xaa Gly Xaa Xaa Ala
 20                 25
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
        30                  35
Xaa Pro Xaa Xaa Xaa Xaa Xaa
                40
Asn Xaa Xaa Asn His Ala Xaa Xaa
        45                  50
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
                55
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
    60                  65
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
            70
Xaa Xaa Xaa Leu Xaa Xaa Xaa
 75                 80
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
            85
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
 90                 95
Xaa Cys Gly Cys Xaa
        100
```

(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(ix) FEATURE:
(A) NAME: hOP-1 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Thr Gly Ser Lys Gln Arg Ser Gln
1 5
Asn Arg Ser Lys Thr Pro Lys Asn Gln
10 15
Glu Ala Leu Arg Met Ala Asn Val Ala
20 25
Glu Asn Ser Ser Ser Asp Gln Arg Gln
30 35
Ala Cys Lys Lys His Glu Leu Tyr Val
40 45
Ser Phe Arg Asp Leu Gly Trp Gln Asp
50
Trp Ile Ile Ala Pro Glu Gly Tyr Ala
55 60
Ala Tyr Tyr Cys Glu Gly Glu Cys Ala
65 70
Phe Pro Leu Asn Ser Tyr Met Asn Ala
75 80
Thr Asn His Ala Ile Val Gln Thr Leu
85 90
Val His Phe Ile Asn Pro Glu Thr Val
95
Pro Lys Pro Cys Cys Ala Pro Thr Gln
100 105
Leu Asn Ala Ile Ser Val Leu Tyr Phe
110 115
Asp Asp Ser Ser Asn Val Ile Leu Lys
120 125
Lys Tyr Arg Asn Met Val Val Arg Ala
130 135
Cys Gly Cys His

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME: mOP-1 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser Thr Gly Gly Lys Gln Arg Ser Gln
1 5
Asn Arg Ser Lys Thr Pro Lys Asn Gln
10 15
Glu Ala Leu Arg Met Ala Ser Val Ala
20 25
Glu Asn Ser Ser Ser Asp Gln Arg Gln
30 35

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ala | Cys | Lys | Lys<br>40 | His | Glu | Leu | Tyr | Val<br>45 |
| Ser | Phe | Arg | Asp | Leu<br>50 | Gly | Trp | Gln | Asp |
| Trp<br>55 | Ile | Ile | Ala | Pro | Glu<br>60 | Gly | Tyr | Ala |
| Ala | Tyr<br>65 | Tyr | Cys | Glu | Gly | Glu<br>70 | Cys | Ala |
| Phe | Pro | Leu<br>75 | Asn | Ser | Tyr | Met | Asn<br>80 | Ala |
| Thr | Asn | His | Ala<br>85 | Ile | Val | Gln | Thr | Leu<br>90 |
| Val | His | Phe | Ile | Asn<br>95 | Pro | Asp | Thr | Val |
| Pro<br>100 | Lys | Pro | Cys | Cys | Ala<br>105 | Pro | Thr | Gln |
| Leu | Asn<br>110 | Ala | Ile | Ser | Val | Leu<br>115 | Tyr | Phe |
| Asp | Asp | Ser<br>120 | Ser | Asn | Val | Ile | Leu<br>125 | Lys |
| Lys | Tyr | Arg | Asn<br>130 | Met | Val | Val | Arg | Ala<br>135 |
| Cys | Gly | Cys | His | | | | | |

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 139 amino acids
  (B) TYPE: amino acids
  (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
  (A) NAME: hOP-2 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ala<br>1 | Val | Arg | Pro | Leu<br>5 | Arg | Arg | Arg | Gln |
| Pro<br>10 | Lys | Lys | Ser | Asn | Glu<br>15 | Leu | Pro | Gln |
| Ala | Asn<br>20 | Arg | Leu | Pro | Gly | Ile<br>25 | Phe | Asp |
| Asp | Val | His<br>30 | Gly | Ser | His | Gly | Arg<br>35 | Gln |
| Val | Cys | Arg | Arg<br>40 | His | Glu | Leu | Tyr | Val<br>45 |
| Ser | Phe | Gln | Asp | Leu<br>50 | Gly | Trp | Leu | Asp |
| Trp<br>55 | Val | Ile | Ala | Pro | Gln<br>60 | Gly | Tyr | Ser |
| Ala | Tyr<br>65 | Tyr | Cys | Glu | Gly | Glu<br>70 | Cys | Ser |
| Phe | Pro | Leu<br>75 | Asp | Ser | Cys | Met | Asn<br>80 | Ala |

Thr Asn His Ala Ile Leu Gln Ser Leu
85 90
Val His Leu Met Lys Pro Asn Ala Val
95
Pro Lys Ala Cys Cys Ala Pro Thr Lys
100 105
Leu Ser Ala Thr Ser Val Leu Tyr Tyr
110 115
Asp Ser Ser Asn Asn Val Ile Leu Arg
120 125
Lys His Arg Asn Met Val Val Lys Ala
130 135
Cys Gly Cys His

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME: mOP-2 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala Ala Arg Pro Leu Lys Arg Arg Gln
1 5
Pro Lys Lys Thr Asn Glu Leu Pro His
10 15
Pro Asn Lys Leu Pro Gly Ile Phe Asp
20 25
Asp Gly His Gly Ser Arg Gly Arg Glu
30 35
Val Cys Arg Arg His Glu Leu Tyr Val
40 45
Ser Phe Arg Asp Leu Gly Trp Leu Asp
50
Trp Val Ile Ala Pro Gln Gly Tyr Ser
55 60
Ala Tyr Tyr Cys Glu Gly Glu Cys Ala
65 70
Phe Pro Leu Asp Ser Cys Met Asn Ala
75 80
Thr Asn His Ala Ile Leu Gln Ser Leu
85 90
Val His Leu Met Lys Pro Asp Val Val
95
Pro Lys Ala Cys Cys Ala Pro Thr Lys
100 105
Leu Ser Ala Thr Ser Val Leu Tyr Tyr
110 115
Asp Ser Ser Asn Asn Val Ile Leu Arg
120 125

Lys His Arg Asn Met Val Val Lys Ala
130 135
Cys Gly Cys His

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(ix) FEATURE:
(A) NAME: CBMP-2A(fx)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser
1 5 10
Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro
15 20
Pro Gly Tyr His Ala Phe Tyr Cys His Gly Glu
25 30
Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser
35 40
Thr Asn His Ala Ile Val Gln Thr Leu Val Asn
45 50 55
Ser Val Asn Ser Lys Ile Pro Lys Ala Cys Cys
60 65
Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu
70 75
Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys
80 85
Asn Tyr Gln Asp Met Val Val Glu Gly Cys Gly
90 95
Cys Arg
100

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 101 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(ix) FEATURE:
(A) NAME: CBMP-2B(fx)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Cys Arg Arg His Ser
1 5
Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
10 15
Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala
20 25

Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu
30 35
Ala Asp His Leu Asn Ser Thr Asn His Ala Ile
40 45
Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser
50 55 60
Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu
65 70
Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu Tyr
75 80
Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met
85 90
Val Val Glu Gly Cys Gly Cys Arg
95 100

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(ix) FEATURE:
(A) NAME: DPP(fx)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser
1 5 10
Asp Val Gly Trp Asp Asp Trp Ile Val Ala Pro
15 20
Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly Lys
25 30
Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser
35 40
Thr Asn His Ala Val Val Gln Thr Leu Val Asn
45 50 55
Asn Asn Asn Pro Gly Lys Val Pro Lys Ala Cys
60 65
Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met
70 75
Leu Tyr Leu Asn Asp Gln Ser Thr Val Val Leu
80 85
Lys Asn Tyr Gln Glu Met Thr Val Val Gly Cys
90 95
Gly Cys Arg
100

(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME: Vgl(fx)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys
 1               5                  10
Asp Val Gly Trp Gln Asn Trp Val Ile Ala Pro
             15                  20
Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly Glu
         25                  30
Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly
     35                  40
Ser Asn His Ala Ile Leu Gln Thr Leu Val His
45                   50                  55
Ser Ile Glu Pro Glu Asp Ile Pro Leu Pro Cys
                60                  65
Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met
             70                  75
Leu Phe Tyr Asp Asn Asn Asp Asn Val Val Leu
         80                  85
Arg His Tyr Glu Asn Met Ala Val Asp Glu Cys
     90                  95
Gly Cys Arg
100
```

(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(ix) FEATURE:
(A) NAME: Vgr-1(fx)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln
 1               5                  10
Asp Val Gly Trp Gln Asp Trp Ile Ile Ala Pro
             15                  20
Xaa Gly Tyr Ala Ala Asn Tyr Cys Asp Gly Glu
         25                  30
Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala
     35                  40
Thr Asn His Ala Ile Val Gln Thr Leu Val His
45                   50                  55
Val Met Asn Pro Glu Tyr Val Pro Lys Pro Cys
                60                  65
Cys Ala Pro Thr Lys Val Asn Ala Ile Ser Val
             70                  75
```

Leu Tyr Phe Asp Asp Asn Ser Asn Val Ile Leu
80 85
Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys
90 95
Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: protein
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(F) TISSUE TYPE: BRAIN

(ix) FEATURE:
(D) OTHER INFORMATION:
/product= "GDF-1 (fx)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly
1 5 10

Trp His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr
15 20 25

Cys Gln Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly
30 35 40

Gly Pro Pro Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His
45 50 55

Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala
60 65 70

Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn
75 80 85

Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu Cys Gly
90 95 100

Cys Arg
105

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Cys Xaa Xaa Xaa Xaa
 1               5
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1822 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS
(F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 49..1341
(D) OTHER INFORMATION:/standard_name= "hOP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
GGTGCGGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCGCG ATG CAC GTG      57
                                                     Met His Val
                                                      1

CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA      105
Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala
     5                   10                  15

CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC      153
Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn
 20                  25                  30                  35

GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG      201
Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg
                 40                  45                  50
```

CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC 249
Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg
55 60 65

CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG 297
Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met
70 75 80

CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC 345
Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly
85 90 95

GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC 393
Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly
100 105 110 115

CCC CCT CTG GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC 441
Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp
120 125 130

ATG GTC ATG AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC 489
Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe
135 140 145

CAC CCA CGC TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC 537
His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile
150 155 160

CCA GAA GGG GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC 585
Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp
165 170 175

TAC ATC CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT 633
Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr
180 185 190 195

CAG GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC 681
Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu
200 205 210

GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC 729
Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp
215 220 225

ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG 777
Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu
230 235 240

GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC 825
Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro
245 250 255

```
AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC    873
Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro
260                 265                 270                 275

TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC    921
Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile
                280                 285                 290

CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC    969
Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro
            295                 300                 305

AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC   1017
Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser
        310                 315                 320

AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC   1065
Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe
    325                 330                 335

CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC   1113
Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala
340                 345                 350                 355

GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG   1161
Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met
                360                 365                 370

AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC   1209
Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn
            375                 380                 385

CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC   1257
Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala
        390                 395                 400

ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA   1305
Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys
    405                 410                 415

TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC        1351
Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420                 425                 430

GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG   1411

GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG   1471

TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC   1531

ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC   1591
```

GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT 1651

CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG 1711

GGCGTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC 1771

CTGTAATAAA TGTCACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A 1822

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 431 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(D) OTHER INFORMATION: /Product="OP1-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
1 5 10 15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
20 25 30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
35 40 45

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
50 55 60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
65 70 75 80

Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
85 90 95

Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
100 105 110

Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
115 120 125

Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
130 135 140

Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
145 150 155 160

```
Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
                165                 170                 175

Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
            180                 185                 190

Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
        195                 200                 205

Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
    210                 215                 220

Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
225                 230                 235                 240

His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
                245                 250                 255

Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
            260                 265                 270

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
        275                 280                 285

Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
    290                 295                 300

Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
305                 310                 315                 320

Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
                325                 330                 335

Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
            340                 345                 350

Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
        355                 360                 365

Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
    370                 375                 380

Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
385                 390                 395                 400

Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
                405                 410                 415

Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
            420                 425                 430
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1873 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: MURIDAE
(F) TISSUE TYPE: EMBRYO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 104..1393
(D) OTHER INFORMATION: /note= "MOP1 (CDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG      60

CGGCGCGGGC CCGGTGCCCC GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC       115
                                                Met His Val Arg
                                                 1

TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT      163
Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro
  5                  10                  15                  20

CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG      211
Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu
                 25                  30                  35

GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG      259
Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg
             40                  45                  50

GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG      307
Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro
         55                  60                  65

CGC CCG CAC CTC CAG GGA AAG CAT AAT TCG GCG CCC ATG TTC ATG TTG      355
Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu
     70                  75                  80

GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG AGC GGG CCG GAC GGA CAG      403
Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly Pro Asp Gly Gln
 85                  90                  95                 100
```

GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT 451
Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro
105 110 115

TTA GCC AGC CTG CAG GAC AGC CAT TTC CTC ACT GAC GCC GAC ATG GTC 499
Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp Met Val
120 125 130

ATG AGC TTC GTC AAC CTA GTG GAA CAT GAC AAA GAA TTC TTC CAC CCT 547
Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro
135 140 145

CGA TAC CAC CAT CGG GAG TTC CGG TTT GAT CTT TCC AAG ATC CCC GAG 595
Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu
150 155 160

GGC GAA CGG GTG ACC GCA GCC GAA TTC AGG ATC TAT AAG GAC TAC ATC 643
Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile
165 170 175 180

CGG GAG CGA TTT GAC AAC GAG ACC TTC CAG ATC ACA GTC TAT CAG GTG 691
Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr Val Tyr Gln Val
185 190 195

CTC CAG GAG CAC TCA GGC AGG GAG TCG GAC CTC TTC TTG CTG GAC AGC 739
Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe Leu Leu Asp Ser
200 205 210

CGC ACC ATC TGG GCT TCT GAG GAG GGC TGG TTG GTG TTT GAT ATC ACA 787
Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp Ile Thr
215 220 225

GCC ACC AGC AAC CAC TGG GTG GTC AAC CCT CGG CAC AAC CTG GGC TTA 835
Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu Gly Leu
230 235 240

CAG CTC TCT GTG GAG ACC CTG GAT GGG CAG AGC ATC AAC CCC AAG TTG 883
Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro Lys Leu
245 250 255 260

GCA GGC CTG ATT GGA CGG CAT GGA CCC CAG AAC AAG CAA CCC TTC ATG 931
Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met
265 270 275

GTG GCC TTC TTC AAG GCC ACG GAA GTC CAT CTC CGT AGT ATC CGG TCC 979
Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg Ser Ile Arg Ser
280 285 290

ACG GGG GGC AAG CAG CGC AGC CAG AAT CGC TCC AAG ACG CCA AAG AAC 1027
Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn
295 300 305

CAA GAG GCC CTG AGG ATG GCC AGT GTG GCA GAA AAC AGC AGC AGT GAC 1075
Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp
310 315 320

CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC TTC CGA GAC 1123
Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp
325 330 335 340

CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC 1171
Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr
345 350 355

TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC 1219
Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala
360 365 370

ACC AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC 1267
Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp
375 380 385

ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT 1315
Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser
390 395 400

GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC ATC CTG AAG AAG TAC AGA 1363
Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg
405 410 415 420

AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCTTCC TGAGACCCTG 1413
Asn Met Val Val Arg Ala Cys Gly Cys His
425 430

ACCTTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG 1473

CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCTGAGCC TTCCCTCACC TCCCAACCGG 1533

AAGCATGTAA GGGTTCCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCCTTCT 1593

GGCACGTGAC GGACAAGATC CTACCAGCTA CCACAGCAAA CGCCTAAGAG CAGGAAAAAT 1653

GTCTGCCAGG AAAGTGTCCA GTGTCCACAT GGCCCCTGGC GCTCTGAGTC TTTGAGGAGT 1713

AATCGCAAGC CTCGTTCAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GGCGCTGGCG 1773

TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAAACCCAT 1833

GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTC 1873

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 430 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(D) OTHER INFORMATION: /product= "mOP1-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
1               5                   10                  15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
            20                  25                  30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
        35                  40                  45

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
    50                  55                  60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
65                  70                  75                  80

Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly
                85                  90                  95

Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr
            100                 105                 110

Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp
        115                 120                 125

Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu
    130                 135                 140

Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser
145                 150                 155                 160

Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr
                165                 170                 175

Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr
            180                 185                 190

Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe
        195                 200                 205
```

Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val
210 215 220

Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His
225 230 235 240

Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile
245 250 255

Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys
260 265 270

Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg
275 280 285

Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys
290 295 300

Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn
305 310 315 320

Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val
325 330 335

Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly
340 345 350

Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser
355 360 365

Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe
370 375 380

Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu
385 390 395 400

Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu
405 410 415

Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420 425 430

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1723 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 490..1696
(D) OTHER INFORMATION: /note= "hOP2 (cDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGGTTG GAGCAGGAGG TGGCACGGCA      60

GGGCTGGAGG GCTCCCTATG AGTGGCGGAG ACGGCCCAGG AGGCGCTGGA GCAACAGCTC     120

CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTCGCCCATC GCCCCTGCGC TGCTCGGACC     180

GCGGCCACAG CCGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCAGT     240

CCGCAGAGTA GCCCCGGCCT CGAGGCGGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG     300

GACAGGTGTC GCGCGGCGGG GCTCCAGGGA CCGCGCCTGA GGCCGGCTGC CCGCCCGTCC     360

CGCCCCGCCC CGCCGCCCGC CGCCCGCCGA GCCCAGCCTC CTTGCCGTCG GGGCGTCCCC     420

AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCCGCTGA GCGCCCCAGC TGAGCGCCCC     480

CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG         528
          Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu
            1               5                  10

GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG CGA CCC CCG CCC      576
Ala Leu Cys Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro
     15                  20                  25

GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG      624
Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln
 30                  35                  40                  45

CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC      672
Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg
                 50                  55                  60

GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG CTC TTC ATG      720
Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met
             65                  70                  75

CTG GAC CTG TAC CAC GCC ATG GCC GGC GAC GAC GAC GAG GAC GGC GCG      768
Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala
         80                  85                  90
```

CCC GCG GAG CGG CGC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC GTT 816
Pro Ala Glu Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val
95 100 105

AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT TGG 864
Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp
110 115 120 125

AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG GTC 912
Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val
130 135 140

ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC CTG CTC 960
Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu
145 150 155

AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC 1008
Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser
160 165 170

AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA GCT 1056
Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala
175 180 185

GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC TGC 1104
Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys
190 195 200 205

TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG GAG 1152
Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu
210 215 220

ACT GAG GAC GGG CAC AGC GTG GAT CCT GGC CTG GCC GGC CTG CTG GGT 1200
Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly
225 230 235

CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC GTG GTC ACT TTC TTC AGG 1248
Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg
240 245 250

GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG GCA GTG AGG CCA CTG AGG 1296
Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg
255 260 265

AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA CTC 1344
Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu
270 275 280 285

CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC TGC 1392
Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys
290 295 300

```
CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG GAC     1440
Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp
            305                 310                 315

TGG GTC ATC GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG GAG     1488
Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu
        320                 325                 330

TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC ATC     1536
Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile
    335                 340                 345

CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG     1584
Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala
350                 355                 360                 365

TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC     1632
Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp
                370                 375                 380

AGC AGC AAC AAC GTC ATC CTG CGC AAA CAC CGC AAC ATG GTG GTC AAG     1680
Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys
            385                 390                 395

GCC TGC GGC TGC CAC T GAGTCAGCCC GCCCAGCCCT ACTGCAG                 1723
Ala Cys Gly Cys His
        400
```

(2) INFORMATION FOR SEQ ID NO:21:

(i)SEQUENCE CHARACTERISTICS:
(A) LENGTH: 402 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii)MOLECULE TYPE: protein

(ix)FEATURE:
(A)OTHER INFORMATION: /product= "hOP2-PP"

(xi)SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
1               5                   10                  15

Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro
            20                  25                  30

Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln Arg Glu Ile
        35                  40                  45
```

Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro
50 55 60

Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu
65 70 75 80

Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu
85 90 95

Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val
100 105 110

Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe
115 120 125

Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala
130 135 140

Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr
145 150 155 160

Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu
165 170 175

Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu
180 185 190

Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu
195 200 205

Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp
210 215 220

Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala
225 230 235 240

Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro
245 250 255

Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln
260 265 270

Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile
275 280 285

Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His
290 295 300

Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile
305 310 315 320

Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe
325 330 335

Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser
340 345 350

Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala
355 360 365

Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn
370 375 380

Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly
385 390 395 400

Cys His

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1926 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: MURIDAE
(F) TISSUE TYPE: EMBRYO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 93..1289
(D) OTHER INFORMATION: /note= "mOP2 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC 50

CCGACCAGCT ACCAGTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT 104
Met Ala Met Arg
1

CCC GGG CCA CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC 152
Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly
5 10 15 20

GGC CAC GGT CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA 200
Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly
25 30 35

```
GCG CGC GAG CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG   248
Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly
            40                  45                  50

CTA CCG GGA CGG CCC CGA CCC CGT GCA CAA CCC GCG GCT GCC CGG CAG   296
Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Ala Arg Gln
        55                  60                  65

CCA GCG TCC GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC   344
Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr
    70                  75                  80

GAT GAC GAC GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC   392
Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp
85                  90                  95                  100

CTG GTC ATG AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC   440
Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly
                105                 110                 115

TAC CAG GAG CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC   488
Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile
            120                 125                 130

CCT GCT GGG GAG GCT GTC ACA GCT GCT GAG TTC CGG ATC TAC AAA GAA   536
Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu
        135                 140                 145

CCC AGC ACC CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA   584
Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu
    150                 155                 160

GTG GTC CAA GAG CAC TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT   632
Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp
165                 170                 175                 180

CTT CAG ACG CTC CGA TCT GGG GAC GAG GGC TGG CTG GTG CTG GAC ATC   680
Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile
                185                 190                 195

ACA GCA GCC AGT GAC CGA TGG CTG CTG AAC CAT CAC AAG GAC CTG GGA   728
Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly
            200                 205                 210

CTC CGC CTC TAT GTG GAA ACC GCG GAT GGG CAC AGC ATG GAT CCT GGC   776
Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly
        215                 220                 225

CTG GCT GGT CTG CTT GGA CGA CAA GCA CCA CGC TCC AGA CAG CCT TTC   824
Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe
    230                 235                 240
```

ATG GTA ACC TTC TTC AGG GCC AGC CAG AGT CCT GTG CGG GCC CCT CGG 872
Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg
245 250 255 260

GCA GCG AGA CCA CTG AAG AGG AGG CAG CCA AAG AAA ACG AAC GAG CTT 920
Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu
265 270 275

CCG CAC CCC AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC 968
Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser
280 285 290

CGC GGC AGA GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGC TTC CGT 1016
Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg
295 300 305

GAC CTT GGC TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC 1064
Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
310 315 320

TAT TAC TGT GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC 1112
Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn
325 330 335 340

GCC ACC AAC CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA 1160
Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
345 350 355

GAT GTT GTC CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC 1208
Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
360 365 370

TCT GTG CTG TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC 1256
Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
375 380 385

CGT AAC ATG GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCCG CCCAGCATCC 1309
Arg Asn Met Val Val Lys Ala Cys Gly Cys His
390 395

TGCTTCTACT ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT 1369

TATCATAGCT CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCCTGCTA 1429

AAATTCTGGT CTTTCCCAGT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC 1489

CTCTCCATCC TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA 1549

ACTGAGAGGT CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC 1609

CTCAGCCCAC AATGGCAAAT TCTGGATGGT CTAAGAAGGC CGTGGAATTC TAAACTAGAT 1669

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GATCTGGGCT CTCTGCACCA TTCATTGTGG CAGTTGGGAC ATTTTTAGGT ATAACAGACA    1729

CATACACTTA GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA    1789

AGAATCAGAG CCAGGTATAG CGGTGCATGT CATTAATCCC AGCGCTAAAG AGACAGAGAC    1849

AGGAGAATCT CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA    1909

AAAAAAAAAC GGAATTC                                                   1926
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 399 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(D) OTHER INFORMATION: /product= "mOP2-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
 1               5                  10                  15

Ala Leu Gly Gly Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln
            20                  25                  30

Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu Ala
        35                  40                  45

Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala
 50                  55                  60                  65

Ala Arg Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala
                70                  75                  80

Met Thr Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg
        85                  90                  95

Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr
    100                 105                 110

Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr
115                 120                 125                 130

Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr
                135                 140                 145
```

```
Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met
            150                 155                 160

Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe
        165                 170                 175

Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu
    180                 185                 190

Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp
195                 200                 205                 210

Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp
                215                 220                 225

Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln
            230                 235                 240

Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala
        245                 250                 255

Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn
    260                 265                 270

Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His
275                 280                 285                 290

Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser
                295                 300                 305

Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr
            310                 315                 320

Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys
        325                 330                 335

Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met
    340                 345                 350

Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser
355                 360                 365                 370

Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg
                375                 380                 385

Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His
            390                 395
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1368 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1368
(D) OTHER INFORMATION:/STANDARD NAME="60A"

(x) PUBLICATION INFORMATION:
(A) AUTHORS: WHARTON, KRISTI A.; THOMSEN, GERALD H.; GELBERT, WILLIAM M.
(B) TITLE: DROSOPHILA 60A GENE...
(C) JOURNAL: PROC. NAT'L ACAD. SCI. USA
(D) VOLUME: 88
(E) RELEVANT RESIDUES IN SEQ ID NO:3: FROM 1 TO 1368
(F) PAGES: 9214-9218
(G) DATE: OCT - 1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
ATG TCG GGA CTG CGA AAC ACC TCG GAG GCC GTT GCA GTG CTC GCC TCC      48
Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser
 1               5                  10                  15

CTG GGA CTC GGA ATG GTT CTG CTC ATG TTC GTG GCG ACC ACG CCG CCG      96
Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro
            20                  25                  30

GCC GTT GAG GCC ACC CAG TCG GGG ATT TAC ATA GAC AAC GGC AAG GAC     144
Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp
        35                  40                  45

CAG ACG ATC ATG CAC AGA GTG CTG AGC GAG GAC GAC AAG CTG GAC GTC     192
Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val
    50                  55                  60

TCG TAC GAG ATC CTC GAG TTC CTG GGC ATC GCC GAA CGG CCG ACG CAC     240
Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His
 65                 70                  75                  80

CTG AGC AGC CAC CAG TTG TCG CTG AGG AAG TCG GCT CCC AAG TTC CTG     288
Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu
                85                  90                  95
```

```
CTG GAC GTC TAC CAC CGC ATC ACG GCG GAG GAG GGT CTC AGC GAT CAG   336
Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln
            100                 105                 110

GAT GAG GAC GAC GAC TAC GAA CGC GGC CAT CGG TCC AGG AGG AGC GCC   384
Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala
        115                 120                 125

GAC CTC GAG GAG GAT GAG GGC GAG CAG CAG AAG AAC TTC ATC ACC GAC   432
Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp
    130                 135                 140

CTG GAC AAG CGG GCC ATC GAC GAG AGC GAC ATC ATC ATG ACC TTC CTG   480
Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu
145                 150                 155                 160

AAC AAG CGC CAC CAC AAT GTG GAC GAA CTG CGT CAC GAG CAC GGC CGT   528
Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg
                165                 170                 175

CGC CTG TGG TTC GAC GTC TCC AAC GTG CCC AAC GAC AAC TAC CTG GTG   576
Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val
            180                 185                 190

ATG GCC GAG CTG CGC ATC TAT CAG AAC GCC AAC GAG GGC AAG TGG CTG   624
Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu
        195                 200                 205

ACC GCC AAC AGG GAG TTC ACC ATC ACG GTA TAC GCC ATT GGC ACC GGC   672
Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly
    210                 215                 220

ACG CTG GGC CAG CAC ACC ATG GAG CCG CTG TCC TCG GTG AAC ACC ACC   720
Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr
225                 230                 235                 240

GGG GAC TAC GTG GGC TGG TTG GAG CTC AAC GTG ACC GAG GGC CTG CAC   768
Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His
                245                 250                 255

GAG TGG CTG GTC AAG TCG AAG GAC AAT CAT GGC ATC TAC ATT GGA GCA   816
Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala
            260                 265                 270

CAC GCT GTC AAC CGA CCC GAC CGC GAG GTG AAG CTG GAC GAC ATT GGA   864
His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly
        275                 280                 285

CTG ATC CAC CGC AAG GTG GAC GAC GAG TTC CAG CCC TTC ATG ATC GGC   912
Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly
    290                 295                 300
```

```
TTC TTC CGC GGA CCG GAG CTG ATC AAG GCG ACG GCC CAC AGC AGC CAC    960
Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His
305                 310                 315                 320

CAC AGG AGC AAG CGA AGC GCC AGC CAT CCA CGC AAG CGC AAG AAG TCG   1008
His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser
                325                 330                 335

GTG TCG CCC AAC AAC GTG CCG CTG CTG GAA CCG ATG GAG AGC ACG CGC   1056
Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg
            340                 345                 350

AGC TGC CAG ATG CAG ACC CTG TAC ATA GAC TTC AAG GAT CTG GGC TGG   1104
Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp
        355                 360                 365

CAT GAC TGG ATC ATC GCA CCA GAG GGC TAT GGC GCC TTC TAC TGC AGC   1152
His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser
    370                 375                 380

GGC GAG TGC AAT TTC CCG CTC AAT GCG CAC ATG AAC GCC ACG AAC CAT   1200
Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His
385                 390                 395                 400

GCG ATC GTC CAG ACC CTG GTC CAC CTG CTG GAG CCC AAG AAG GTG CCC   1248
Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro
                405                 410                 415

AAG CCC TGC TGC GCT CCG ACC AGG CTG GGA GCA CTA CCC GTT CTG TAC   1296
Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr
            420                 425                 430

CAC CTG AAC GAC GAG AAT GTG AAC CTG AAA AAG TAT AGA AAC ATG ATT   1344
His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile
        435                 440                 445

GTG AAA TCC TGC GGG TGC CAT TGA                                   1368
Val Lys Ser Cys Gly Cys His
    450                 455
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 455 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser
1 5 10 15

Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro
20 25 30

Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp
35 40 45

Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val
50 55 60

Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His
65 70 75 80

Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu
85 90 95

Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln
100 105 110

Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala
115 120 125

Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp
130 135 140

Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu
145 150 155 160

Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg
165 170 175

Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val
180 185 190

Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu
195 200 205

Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly
210 215 220

Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr
225 230 235 240

Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His
245 250 255

Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala
260 265 270

His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly
        275                 280                 285

Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly
    290                 295                 300

Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His
305                 310                 315                 320

His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser
                325                 330                 335

Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg
            340                 345                 350

Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp
        355                 360                 365

His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser
    370                 375                 380

Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His
385                 390                 395                 400

Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro
                405                 410                 415

Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr
            420                 425                 430

His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile
        435                 440                 445

Val Lys Ser Cys Gly Cys His
    450                 455

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) ORIGINAL SOURCE:
(A) ORGANISM: Homo Sapiens

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /note="BMP3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

(i)SEQUENCE CHARACTERISTICS:
(A) LENGTH: 104 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii)MOLECULE TYPE: protein

(ix)FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..104
(D) OTHER INFORMATION: /note="BMP3"

(xi)SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser
1               5                   10                  15

Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Try Cys Ser Gly
            20                  25                  30

Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His Ala
        35                  40                  45

Thr Ile Gln Ser Ile Val Ala Arg Ala Val Gly Val Val Pro Gly Ile
    50                  55                  60

Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu
65                  70                  75                  80

Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met
                85                  90                  95

Thr Val Glu Ser Cys Ala Cys Arg
            100
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /note= "BMP5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln
1               5                   10                  15

Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly
            20                  25                  30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
        35                  40                  45

Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys
    50                  55                  60

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65                  70                  75                  80

Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
                85                  90                  95

Arg Ser Cys Gly Cys His
            100
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /note= "BMP6"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Cys Arg Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln
1               5                   10                  15
```

Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
20 25 30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
35 40 45

Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys
50 55 60

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65 70 75 80

Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Trp Met Val Val
85 90 95

Arg Ala Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /label= OPX
/note= "WHEREIN XAA AT EACH POS'N IS INDEPENDENTLY SELECTED FROM THE RESIDUES OCCURRING AT THE CORRESPONDING POS'N IN THE C-TERMINAL SEQUENCE OF MOUSE OR HUMAN OP1 OR OP2 (SEE SEQ. ID NOS. 5,6,7 and 8 or 16,18,20 and 22.)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
1 5 10 15

Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
20 25 30

Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
35 40 45

Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
50 55 60

Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
65 70 75 80

Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
85 90 95

Xaa Ala Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:30:

(i)SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii)MOLECULE TYPE: protein
(ix)FEATURE:
(A) NAME: Generic Sequence 5
(D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi)SEQUENCE DESCRIPTION: SEQ ID NO:30:

Leu Xaa Xaa Xaa Phe
1 5
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
10
Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
15 20
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
25 30
Xaa Pro Xaa Xaa Xaa Xaa Xaa
35
Xaa Xaa Xaa Asn His Ala Xaa Xaa
40 45
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
55 60
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
65
Xaa Xaa Xaa Leu Xaa Xaa Xaa
70 75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
80
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
85 90
Xaa Cys Xaa Cys Xaa
95

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(ix) FEATURE:
(A) NAME: Generic Sequence 6
(D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe
 1               5                   10
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                15
Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
 20                  25
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
        30                  35
Xaa Pro Xaa Xaa Xaa Xaa Xaa
                40
Xaa Xaa Xaa Asn His Ala Xaa Xaa
        45                  50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                55
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
    60                  65
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
            70
Xaa Xaa Xaa Leu Xaa Xaa Xaa
 75                  80
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
            85
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
 90                  95
Xaa Cys Xaa Cys Xaa
        100
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1238 base pairs, 372 amino acids
(B) TYPE: nucleic acid, amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) ORIGINAL SOURCE:
(A) ORGANISM: human
(F) TISSUE TYPE: BRAIN

(iv) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:
(D) OTHER INFORMATION:
/product= "GDF-1"
/note= "GDF-1 CDNA"

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Lee, Se-Jin
(B) TITTLE: Expression of Growth/Differentiation Factor 1
(C) JOURNAL: Proc. Nat'l Acad. Sci.
(D) VOLUME: 88
(E) RELEVANT RESIDUES: 1-1238
(F) PAGES: 4250-4254
(G) DATE: May-1991
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
GGGGACACCG GCCCCGCCCT CAGCCCACTG GTCCCGGGCC GCCGCGGACC CTGCGCACTC      60

TCTGGTCATC GCCTGGGAGG AAG ATG CCA CCG CCG CAG CAA GGT CCC TGC GGC     113
                          Met Pro Pro Pro Gln Gln Gly Pro Cys Gly
                           1               5                  10

CAC CAC CTC CTC CTC CTC CTG GCC CTG CTG CTG CCC TCG CTG CCC           158
His His Leu Leu Leu Leu Leu Ala Leu Leu Leu Pro Ser Leu Pro
                15                  20                  25

CTG ACC CGC GCC CCC GTG CCC CCA GGC CCA GCC GCC GCC CTG CTC           203
Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu
                30                  35                  40

CAG GCT CTA GGA CTG CGC GAT GAG CCC CAG GGT GCC CCC AGG CTC           248
Gln Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu
                45                  50                  55

CGG CCG GTT CCC CCG GTC ATG TGG CGC CTG TTT CGA CGC CGG GAC           293
Arg Pro Val Pro Pro Val Met Trp Arg Leu Phe Arg Arg Arg Asp
                60                  65                  70

CCC CAG GAG ACC AGG TCT GGC TCG CGG CGG ACG TCC CCA GGG GTC           338
Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg Thr Ser Pro Gly Val
                75                  80                  85

ACC CTG CAA CCG TGC CAC GTG GAG GAG CTG GGG GTC GCC GGA AAC           383
Thr Leu Gln Pro Cyc His Val Glu Glu Leu Gly Val Ala Gly Asn
                90                  95                 100
```

ATC GTG CGC CAC ATC CCG GAC CGC GGT GCG CCC ACC CGG GCC TCG 428
Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr Arg Ala Ser
105 110 115

GAG CCT GTC TCG GCC GCG GGG CAT TGC CCT GAG TGG ACA GTC GTC 473
Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr Val Val
120 125 130

TTC GAC CTG TCG GCT GTG GAA CCC GCT GAG CGC CCG AGC CGG GCC 518
Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg Ala
135 140 145

CGC CTG GAG CTG CGT TTC GCG GCG GCG GCG GCG GCA GCC CCG GAG 563
Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Ala Pro Glu
150 155 160

GGC GGC TGG GAG CTG AGC GTG GCG CAA GCG GGC CAG GGC GCG GGC 608
Gly Gly Trp Glu Leu Ser Val Ala Gln Ala Gly Gln Gly Ala Gly
165 170 175

GCG GAC CCC GGG CCG GTG CTG CTC CGC CAG TTG GTG CCC GCC CTG 653
Ala Asp Pro Gly Pro Val Leu Leu Arg Gln Leu Val Pro Ala Leu
180 185 190

GGG CCG CCA GTG CGC GCG GAG CTG CTG GGC GCC GCT TGG GCT CGC 698
Gly Pro Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg

195 200 205

AAC GCC TCA TGG CCG CGC AGC CTC CGC CTG GCG CTG GCG CTA CGC 743
Asn Ala Ser Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg
210 215 220

CCC CGG GCC CCT GCC GCC TGC GCG CGC CTG GCC GAG GCC TCG CTG 788
Pro Arg Ala Pro Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu
225 230 235

CTG CTG GTG ACC CTC GAC CCG CGC CTG TGC CAC CCC CTG GCC CGG 833
Leu Leu Val Thr Leu Asp Pro Arg Leu Cys His Pro Leu Ala Arg
240 245 250

CCG CGG CGC GAC GCC GAA CCC GTG TTG GGC GGC GGC CCC GGG GGC 878
Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly
255 260 265

GCT TGT CGC GCG CGG CGG CTG TAC GTG AGC TTC CGC CAG GTG GGC 923
Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly
270 275 280

TGG CAC CGC TGG GTC ATC GCG CCG CGC CCC TTC CTG GCC AAC TAC 968
Trp His Arg Trp Val Ile Arg Pro Arg Gly Phe Leu Ala Asn Tyr
285 290 295

```
TGC CAG GGT CAG TGC GCG CTG CCC GTC GCG CTG TCG GGG TCC GGG   1013
Cys Gln Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly
                300                 305                 310

GGG CCG CCG GCG CTC AAC CAC GCT GTG CTG CGC GCG CTC ATG CAC   1058
Gly Pro Pro Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His
                315                 320                 325

GCG GCC GCC CCG GGA GCC GCC GAC CTG CCC TGC TGC GTG CCC GCG   1103
Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala
                330                 335                 340

CGC CTG TCG CCC ATC TCC GTG CTC TTC TTT GAC AAC AGC GAC AAC   1148
Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn
                345                 350                 355

GTG GTG CTG CGG CAG TAT GAG GAC ATG GTG GTG GAC GAG TGC GGC   1193
Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu Cys Gly
                360                 365                 370

TGC CGC TAACCCGGGG CGGGCAGGGA CCCGGGCCCA ACAATAAATG CCGCGTGG  1238
Cys Arg
    372
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 372 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: human
 (F) TISSUE TYPE: BRAIN

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION:
 (D) OTHER INFORMATION: /function=
  /product= "GDF-1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Met Pro Pro Pro Gln Gln Gly Pro Cys Gly
1               5                   10
```

His His Leu Leu Leu Leu Leu Ala Leu Leu Leu Pro Ser Leu Pro
15 20 25

Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu
30 35 40

Gln Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu
45 50 55

Arg Pro Val Pro Pro Val Met Trp Arg Leu Phe Arg Arg Arg Asp
60 65 70

Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg Thr Ser Pro Gly Val
75 80 85

Thr Leu Gln Pro Cyc His Val Glu Glu Leu Gly Val Ala Gly Asn
90 95 100

Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr Arg Ala Ser
105 110 115

Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr Val Val
120 125 130

Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg Ala
135 140 145

Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Ala Pro Glu
150 155 160

Gly Gly Trp Glu Leu Ser Val Ala Gln Ala Gly Gln Gly Ala Gly
165 170 175

Ala Asp Pro Gly Pro Val Leu Leu Arg Gln Leu Val Pro Ala Leu
180 185 190

Gly Pro Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg
195 200 205

Asn Ala Ser Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg
210 215 220

Pro Arg Ala Pro Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu
225 230 235

Leu Leu Val Thr Leu Asp Pro Arg Leu Cys His Pro Leu Ala Arg
240 245 250

Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly
255 260 265

Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly
270 275 280

Trp His Arg Trp Val Ile Arg Pro Arg Gly Phe Leu Ala Asn Tyr
285 290 295

Cys Gln Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly
300 305 310

Gly Pro Pro Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His
315 320 325

Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala
330 335 340

Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn
345 350 355

Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu Cys Gly
360 365 370

Cys Arg
372

What is claimed is:

1. A method for alleviating the tissue destructive effects associated with the inflammatory response to tissue injury in a mammal, the method comprising the step of:

   providing to the injured tissue a therapeutically effective concentration of a morphogen sufficient to substantially inhibit or reduce the tissue damage resulting from said inflammatory response.

2. The method of claim 1 where said step of providing a therapeutically effective morphogen concentration to said injured tissue comprises the step of administering a therapeutically effective concentration of a morphogen to said mammal.

3. The method of claim 1 where said step of providing a therapeutically effective morphogen concentration to said injured tissue comprises the step of administering to said mammal an agent that stimulates in vivo a therapeutically effective concentration of an endogenous morphogen.

4. The method of claim 1 wherein said step of providing a therapeutically effective concentration of a morphogen is conducted prior to reduction or interruption of blood flow to the tissue.

5. The method of claim 1 wherein said step of providing a therapeutically effective concentration of a morphogen is conducted after reduction or interruption of blood flow to the tissue and before reperfusion.

6. The method of claim 1 wherein said step of administering a therapeutically effective amount of a morphogen is conducted following ischemia-reperfusion injury.

7. The method of claim 1 wherein said said step of administering a therapeutically effective amount of a morphogen is conducted following hyperoxia injury.

8. The method of claim 1 wherein said morphogen is provided to said tissue prior to said tissue injury.

9. The method of claim 1 wherein said step of providing a therapeutically effective concentration of a morphogen is conducted prior to ischemia-reperfusion injury.

10. The method of claim 1 wherein said tissue damage results from an abnormal immune response in said mammal.

11. The method of claim 1 wherein said tissue damage is associated with an inflammatory disease.

12. The method of claim 11 wherein said inflammatory disease is an autoimmune disease.

13. The method of claim 11 wherein said inflammatory disease comprises arthritis, psoriasis, dermatitis or diabetes.

14. The method of claim 13 wherein said arthritis is rhematoid, degenerative or psoriatic arthritis.

15. The method of claim 11 wherein said inflammatory disease comprises an airway inflammation in a mammal.

16. The method of claim 15 wherein said airway inflammation comprises chronic bronchitis, emphysema, idiopathic pulmonary fibrosis or asthma.

17. The method of claim 11 wherein inflammatory disease comprises a generalized acute inflammatory response.

18. The method of claim 17 wherein said inflammatory disease comprises adult respiratory distress syndrome.

19. The method of claim 1 wherein said tissue damage is to a transplanted organ or tissue.

20. A method for reducing tissue damage associated with ischemia-reperfusion injury in a human, the method comprising the step of:

providing to the injured tissue a therapeutic concentration of a morphogen sufficient to alleviate the damage associated with said injury.

21. A method for reducing the tissue damage associated with hyperoxia injury in a human, the method comprising the step of:

providing to the injured tissue a therapeutic concentration of a morphogen sufficient to alleviate the damage associated with said injury.

22. The method of claim 20 or 21 wherein said step of providing a therapeutically effective morphogen concentration to said injured tissue comprises the step of administering a therapeutically effective concentration of a morphogen to said mammal.

23. The method of claim 20 or 21 wherein said step of providing a therapeutically effective morphogen concentration to said injured tissue comprises the step of administering to said mammal an agent that stimulates <u>in vivo</u> a therapeutically effective concentration of an endogenous morphogen.

24. The method of claim 1, 20 or 21 wherein said tissue is lung tissue, cardiac tissue, hepatic tissue or renal tissue.

25. The method of claim 6, 9 or 20 wherein said ischemic-reperfusion injury results from cardiac arrest, preliminary occlusion, arterial occlusion, coronary occlusion or occlusive stroke.

26. The method of claim 1, 20 or 21 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).

27. The method of claim 26 wherein said morphogen comprises an amino acid sequence sharing a last 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, BMP3(fx), BMP5(fx), BMP6(fx), Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).

28. The method of claim 1, 20 or 21 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).

29. The method of claim 28 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).

30. The method of claim 29 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.

31. The method of claim 1, 20 or 21 wherein said morphogen comprises an amino acid sequence defined by Generic Sequences 1, 2, 3, 4, 5 or 6 (Seq. ID Nos. 1, 2, 3, 4, 30 or 31).

32. The method of claim 1, 20 or 21 wherein said morphogen comprises an amino acid sequence defined by OPX (Seq. ID No. 29).

33. A method for reducing the ischemic-reperfusion injury associated with the interruption of blood flow to an organ in a clinical procedure, the method comprising the step of providing a therapeutic concentration of a morphogen to said organ prior to the interruption of blood flow.

34. A method for reducing the tissue injury associated with the reduction or interruption of blood flow to an organ or tissue in a clinical procedure, the method comprising the step of providing a therapeutic concentration of a morphogen to said organ or tissue after the reduction or interruption of blood flow to said organ or tissue.

35. The method of claim 33 or 34 wherein said clinical procedure is a carotid enterectomy, a coronary artery bypass, a tissue grafting procedure, an organ transplant, or a fibrinolytic therapy.

36. The method of claim 1, 33 or 34 wherein said morphogen is administered parenterally.

37. The method of claim 1, 33 or 34 wherein said morphogen is administered prophylactically.

38. A pharmaceutical composition for use in alleviating the injury associated with tissue exposure to toxic oxygen concentrations comprising a therapeutically effective amount of a morphogen in admixture with a free oxygen radical inhibiting agent or an anticoagulent.

39. A pharmaceutical composition for topical administration comprising a therapeutically effective concentration of a morphogen in admixture with a dermatologically acceptable carrier.

40. A pharmaceutical composition for topical administration to a tissue comprising a therapeutically effective concentration of a morphogen dispersed in a biocompatible, non-irritating tissue surface adhesive.

41. The composition of claim 40 wherein said adhesieve comprises hydroxypropylcellulose.

42. The composition of claim 38, 39 or 40 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).

43. The composition of claim 42 wherein said morphogen comprises an amino acid sequence sharing a last 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, BMP3(fx), BMP5(fx), BMP6(fx), Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).

44. The composition of claim 38, 39 or 40 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).

45. The composition of claim 44 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).

46. The method of claim 45 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.

47. The composition of claim 38, 39 or 40 wherein said morphogen comprises an amino acid sequence defined by Generic Sequences 1, 2, 3, 4, 5 or 6 (Seq. ID Nos. 1, 2, 3, 4, 30 or 31).

48. The composition of claim 38, 39 or 40 wherein said morphogen comprises an amino acid sequence defined by OPX (Seq. ID No. 29).

49. A method of enhancing the viability of an organ or tissue to be transplanted in a mammal, the method of comprising the step of:

providing a therapeutically effective concentration of a morphogen to said tissue or organ to be transplanted.

50. The method of claim 49 wherein said therapeutically effective concentration is sufficient to substantially inhibit reperfusion injury to said tissue or organ.

51. The method of claim 49 wherein said morphogen is provided to said tissue or organ prior to reperfusion injury.

52. The method of claim 49 wherein said morphogen is provided to said tissue or organ prior to removal of said tissue or organ from the donor.

53. The method of claim 49 wherein said organ is placed in an organ preservation solution containing said morphogen or a morphogen-stimulating agent after removal of said organ from the donor and prior to transplantation in the recipient.

54. The method of claim 49 wherein said organ is selected from the group consisting of lung, heart, kidney, liver or pancreas.

55. The method of claim 49 wherein said living tissue comprises skin, bone marrow or gastrointestinal mucosa tissue.

56. A method for protecting a living tissue or transplant organ from the tissue destructive effects associated with the inflammatory response in a mammal, the method comprising the step of:

providing to said tissue or organ a therapeutically effective concentration of a morphogen.

57. A method of protecting a living tissue or transplanted organ from ischemia-reperfusion injury in a mammal, the method comprising the step of:

providing to said tissue or organ a therapeutically effective concentration of a morphogen, said concentration being sufficient to substantially inhibit or reduce the tissue damage associated with ischemia-reperfusion injury.

58. The method of claim 49, 56 or 57 wherein said step of providing a therapeutically effective morphogen concentration to said injured tissue comprises the step of administering a therapeutically effective concentration of a morphogen to said mammal.

59. The method of claim 49, 56 or 57 wherein said step of providing a therapeutically effective morphogen concentration to said injured tissue comprises the step of administering to said mammal an agent that stimulates <u>in vivo</u> a therapeutically effective concentration of an endogenous morphogen.

60. A composition useful as a living cell or living tissue preservation solution comprising:

a fluid formulation having as osmotic pressure substantially equivalent to the osmotic pressure of living mammalian cells in admixture with

a therapeutically effective concentration of a morphogen or morphogen-stimulating agent, said concentration being sufficient to protect living cell or tissue from the tissue destructive effects associated with the inflammatory response in a mammal when exposed to said cells or tissue.

61. The preservation solution of claim 60 wherein said therapeutically effective concentration is sufficient to substantially inhibit or reduce the tissue damage associated with ischemia-reperfusion injury.

62. The preservation solution of claim 60 wherein said formulation further comprises a sugar.

63. The preservation solution of claim 60 wherein said formulation further comprises an anticoagulant or a free oxygen radical inhibiting agent.

64. The invention of claim 49, 56, 57 or 60 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).

65. A composition useful in a treatment method to alleviate tissue damage associated with the inflammatory response in a mammal, the composition comprising a therapeutically effective concentration of a morphogen or morphogen-stimulating agent.

66. The composition of claim 65 wherein said tissue damage is associated with ischemia-reperfusion injury or hyperoxia injury.

67. The composition of claim 65 wherein said tissue damage is to lung, cardiac, renalor hepatic tissue.

68. The composition of claim 65 wherein said tissue damage is to a transplanted organ or tissue.

AMENDED CLAIMS

[received by the International Bureau
on 10 February 1993 (10.02.93);
original claims 46 and 49 amended;
remaining claims unchanged (1 page)]

44. The composition of claim 38, 39 or 40 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).

45. The composition of claim 44 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).

46. The composition of claim 45 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.

47. The composition of claim 38, 39 or 40 wherein said morphogen comprises an amino acid sequence defined by Generic Sequences 1, 2, 3, 4, 5 or 6 (Seq. ID Nos. 1, 2, 3, 4, 30 or 31).

48. The composition of claim 38, 39 or 40 wherein said morphogen comprises an amino acid sequence defined by OPX (Seq. ID No. 29).

49. A method of enhancing the viability of an organ or tissue to be transplanted in a mammal, the method comprising the step of:

providing a therapeutically effective concentration of a morphogen to said tissue or organ to be transplanted.

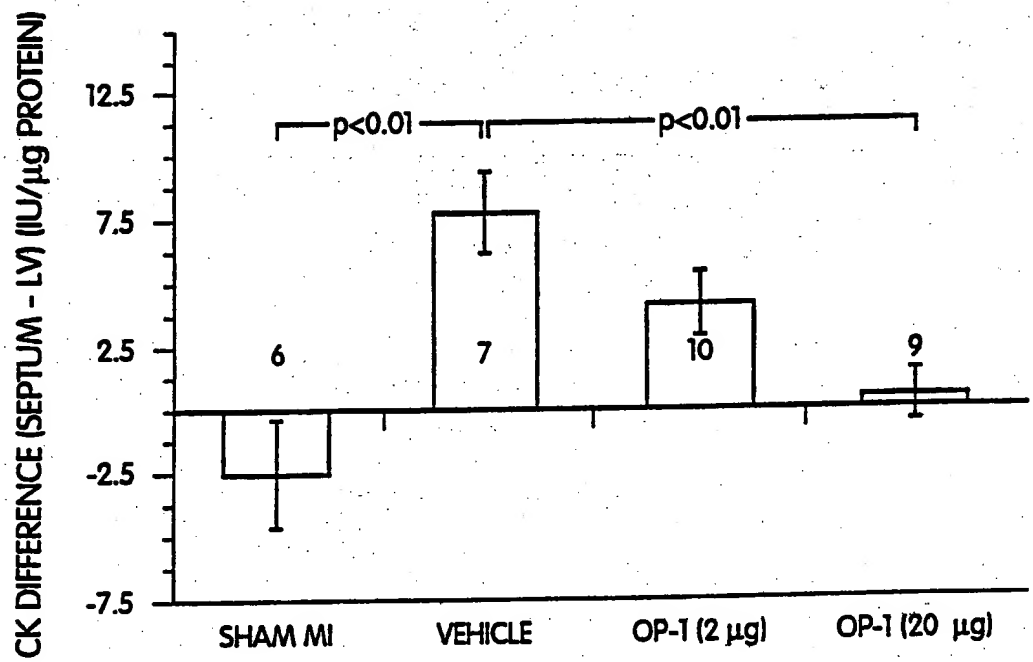
CK DIFFERENCE (SEPTUM - LV) (IU/µg PROTEIN)
12.5
7.5
2.5
-2.5
-7.5
p<0.01
p<0.01
6
7
10
9
SHAM MI
VEHICLE
OP-1 (2 µg)
OP-1 (20 µg)

Fig. 1

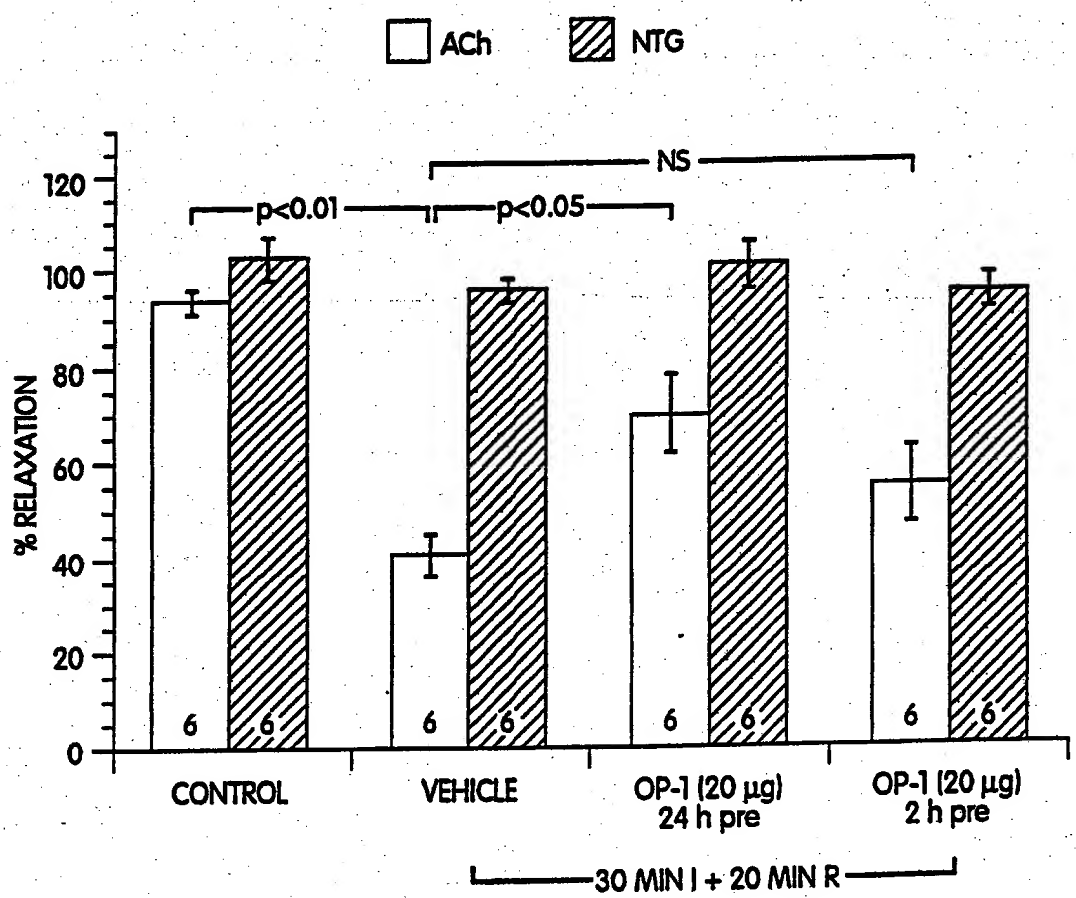
ACh
NTG
NS
p<0.01
p<0.05
120
100
80
60
40
20
0
% RELAXATION
6
CONTROL
VEHICLE
OP-1 (20 µg) 24 h pre
OP-1 (20 µg) 2 h pre
30 MIN I + 20 MIN R

Fig 2

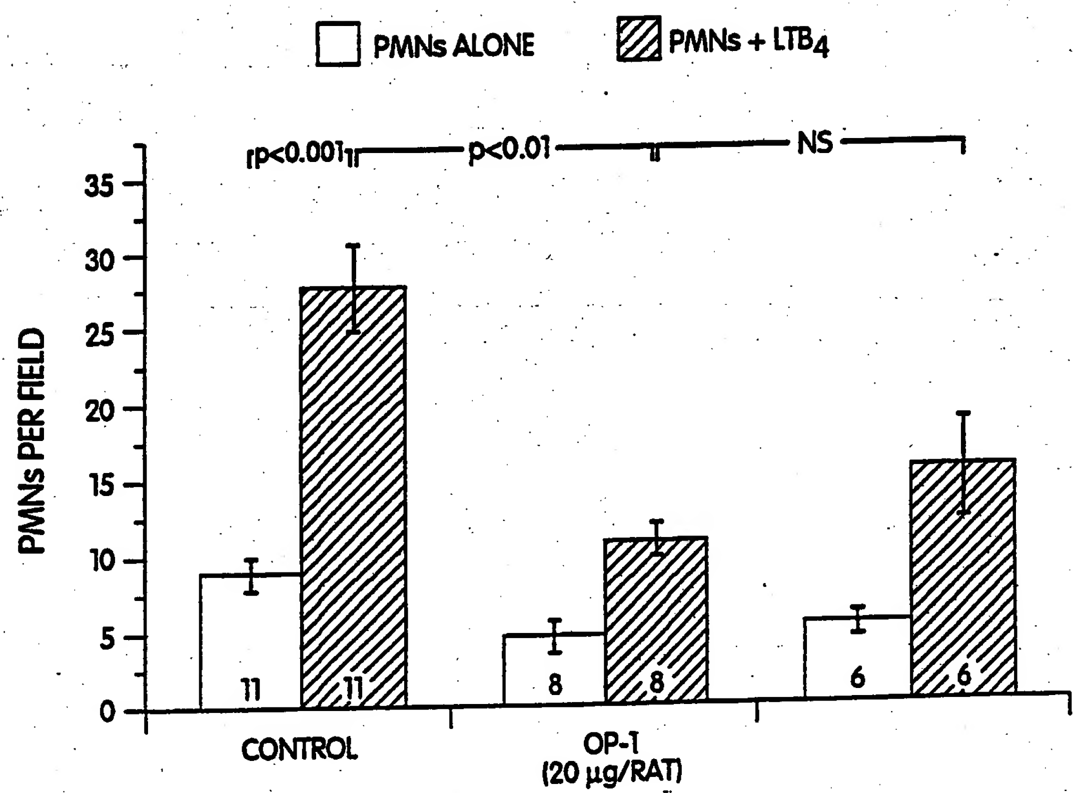
PMNs ALONE
PMNs + LTB4
p<0.001
p<0.01
NS
PMNs PER FIELD
35
30
25
20
15
10
5
0
11
11
8
8
6
6
CONTROL
OP-1
(20 µg/RAT)

Fig. 3

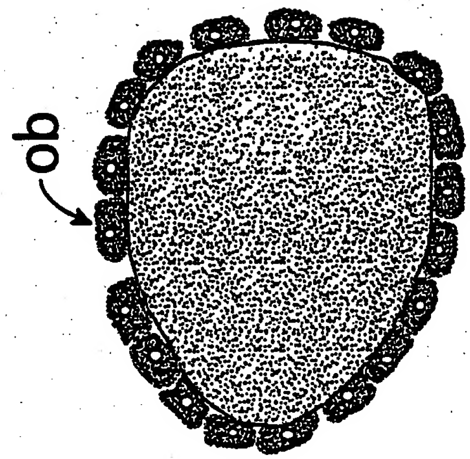


Fig. 4B

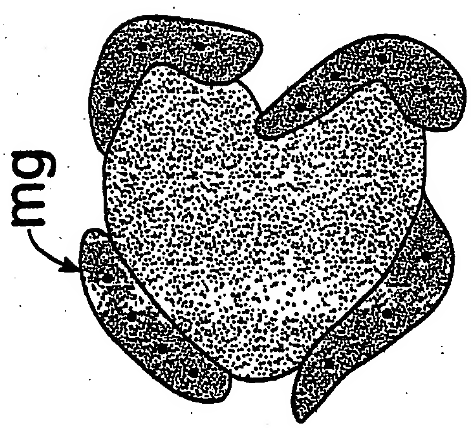


Fig. 4A

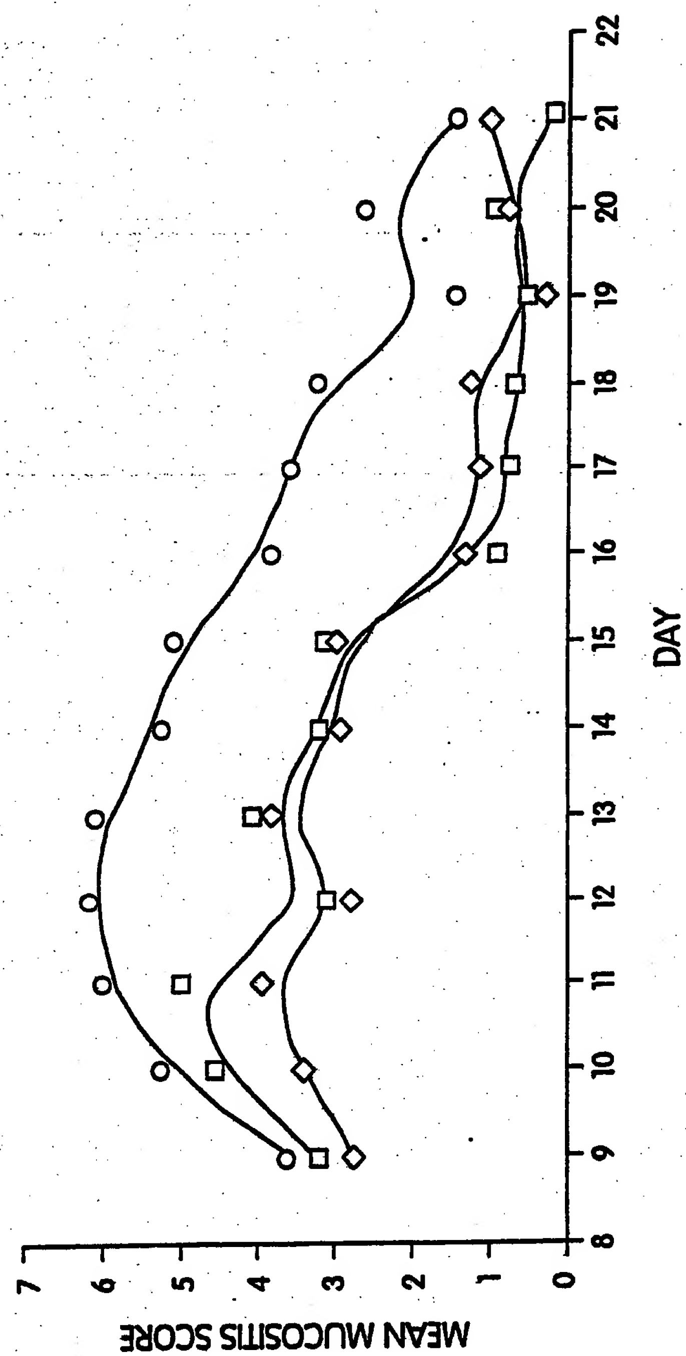
MEAN MUCOSITIS SCORE
0
1
2
3
4
5
6
7
DAY
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

Fig. 5

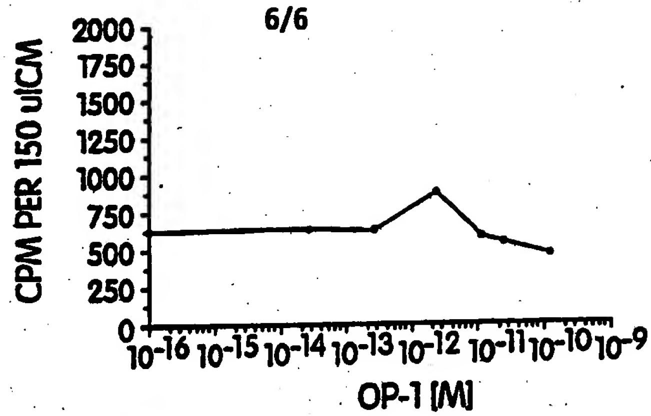


Fig. 6A

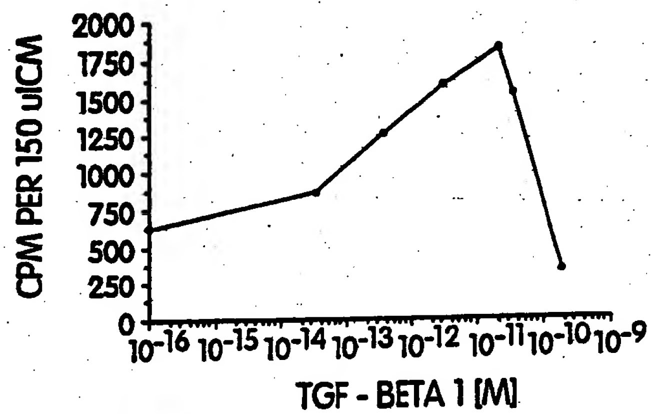


Fig. 6B

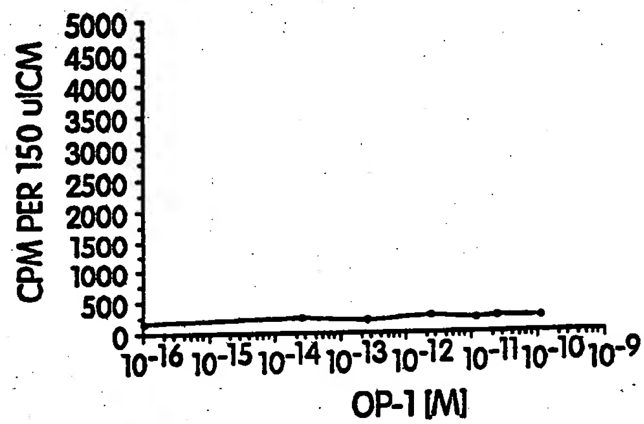


Fig. 6C

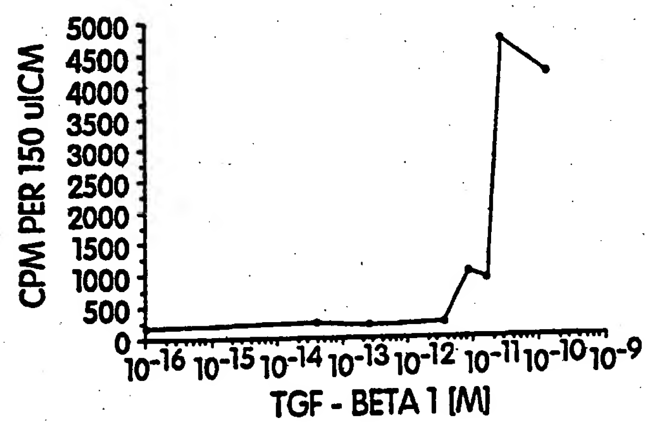


Fig. 6D

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/07358

**I. CLASSIFICATION OF SUBJECT MATTER** (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K37/02; A01N1/02

**II. FIELDS SEARCHED**

Minimum Documentation Searched[7]

| Classification System | Classification Symbols |
| --- | --- |
| Int.Cl. 5 | A61K ; C07K |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

**III. DOCUMENTS CONSIDERED TO BE RELEVANT**[9]

| Category ° | Citation of Document, [11] with indication, where appropriate, of the relevant passages [12] | Relevant to Claim No.[13] |
| --- | --- | --- |
| X | SCIENCE vol. 249, no. 4964, 6 July 1990, LANCASTER, PA US pages 61 - 64 LEFER A.M. ET AL 'Mediation of cardioprotection by Transforming growth factor-beta' cited in the application see the whole document --- | 1-2,4-9, 20-22, 25, 33-37, 57-58, 65-68 |
| X | PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 88, no. 7, April 1991, WASHINGTON US pages 2918 - 2921 KURUVILLA, A.P. 'Protective effect of transforming growth factor beta1 on experimental autoimmune diseases in mice' cited in the application see the whole document --- -/-- | 1-2, 10-19, 36-37, 56,58,65 |

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

**IV. CERTIFICATION**

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
| --- | --- |
| 19 NOVEMBER 1992 | 18. 12. [illegible] |
| **International Searching Authority** | **Signature of Authorized Officer** |
| EUROPEAN PATENT OFFICE | FERNANDEZ Y BRA F. |

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

| Category ° | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No. |
|---|---|---|
| X | EP,A,0 269 408 (GENENTECH INC.) 1 June 1988<br>see the whole document | 1-2, 10-19, 36-37, 56,58,65 |
| X | WO,A,9 000 900 (AMGEN INC.) 8 February 1990<br>see the whole document | 1-2, 10-19, 36-37, 56,58,65 |
| E | WO,A,9 215 323 (CREATIVE BIOMOLECULES) 17 September 1992<br>see page 6, line 1 - page 7, line 27<br>see page 77 - page 119 | 1-6, 8-12, 15-17, 20, 22-37, 56-59, 64-67 |
| X | WO,A,9 105 802 (CREATIVE BIOMOLECULES) 2 May 1991<br>see page 20, line 19 - line 31<br>see page 59 - page 80 | 1-2, 10-13, 26-32, 36-37,65 |
| X,P | WO,A,9 207 073 (CREATIVE BIOMOLECULES) 30 April 1992<br>see page 11, line 3 - line 15<br>see page 41 - page 60 | 1-2, 10-13, 26-29, 31-32, 36-37,65 |
| X | WO,A,8 909 788 (CREATIVE BIOMOLECULES) 19 October 1989 cited in the application<br>see page 6 - page 15 | 1-2, 10-13, 26-29, 31-32, 36-37,65 |
| A | EMBO JOURNAL vol. 9, no. 7, 1990, EYNSHAM, OXFORD GB pages 2085 - 2093 ÖZKAYNAK, E. ET AL 'OP-1 cDNA encodes an osteogenic protein in the TGF-beta family'<br>see the whole document | 1-68 |

International application No.

PCT/US 92/ 07358

# INTERNATIONAL SEARCH REPORT

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

   see annex

2. [X] Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

   see annex

3. [ ] Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Remark: Although claims 1-37,49-52 (partially, when the method is carried out in vivo), 54-57 (partially, when the method is carried out in vivo), 58 - 59,64 (partially, according to the method of claims 49,56 or 57) are directed to a method of treatment of the human or animal body the search has been carried out and based on the alleged effects of the composition.

OBSCURITIES, INCONSISTENCIES, CONTRADICTIONS, LACK OF CONCISENESS; LACK OF READY COMPREHENSIBILITY)

(ART. 6 PCT)

REASON:

1. Claim 46 has been understood as being dependant of claim 45. Therefore claim 46 should read: "The composition of claim 45, wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOPI), including allelic and species variants thereof.

2. In view of the extremely large number of compounds used in the methods and compositions of claims 26-29, 31 (in as far as seq. ID 1 to 4 and 30-31), 42-45,47 (in as far as seq. ID 1 to 4 and 30-31), 64, the search division considers that it is not economically reasonable to draw up a search report for the methods using, or the compositions comprising all the compounds defined in the claims. The search has therefore been limited, on the basis of the examples and claims, to the methods using, or the compositions comprising the seq. ID no. 5 to 29, 32 and 33 (Art. 17 (2) (a)(ii) and (b) PCT.

3. The term "morphogen" is not concise.

   Therefor, and for the same reaons as given in paragraph 2 above, it has been understood as being one of the proteins defines in seq. ID 5 to 29, 32 or 33. (Art. 6 PCT and Art. 17/2)(a)(ii) and (b) PCT)

**ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.** US 9207358
SA 64364

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 19/11/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| EP-A-0269408 | 01-06-88 | JP-A- 63211234 | 02-09-88 |
| WO-A-9000900 | 08-02-90 | AU-A- 4056089 | 19-02-90 |
| WO-A-9215323 | 17-09-92 | None | |
| WO-A-9105802 | 02-05-91 | AU-A- 6648190 | 16-05-91 |
| | | CA-A- 2042577 | 18-04-91 |
| | | EP-A- 0448704 | 02-10-91 |
| | | JP-T- 4502336 | 23-04-92 |
| | | CA-A- 2027259 | 18-04-91 |
| WO-A-9207073 | 30-04-92 | AU-A- 8900091 | 20-05-92 |
| WO-A-8909788 | 19-10-89 | US-A- 4968590 | 06-11-90 |
| | | US-A- 5011691 | 30-04-91 |
| | | AU-B- 628050 | 10-09-92 |
| | | AU-A- 3444989 | 03-11-89 |
| | | AU-B- 618357 | 19-12-91 |
| | | AU-A- 3530589 | 03-11-89 |
| | | EP-A- 0372031 | 13-06-90 |
| | | EP-A- 0362367 | 11-04-90 |
| | | JP-T- 3500655 | 14-02-91 |
| | | JP-T- 3502579 | 13-06-91 |
| | | WO-A- 8909787 | 19-10-89 |
| | | US-A- 5108753 | 28-04-92 |
| | | AU-B- 627850 | 03-09-92 |
| | | AU-A- 5174790 | 26-09-90 |
| | | EP-A- 0411105 | 06-02-91 |
| | | JP-T- 3504736 | 17-10-91 |
| | | WO-A- 9010018 | 07-09-90 |
| | | US-A- 4975526 | 04-12-90 |